JOURNAL OF DAIRY SCIENCE

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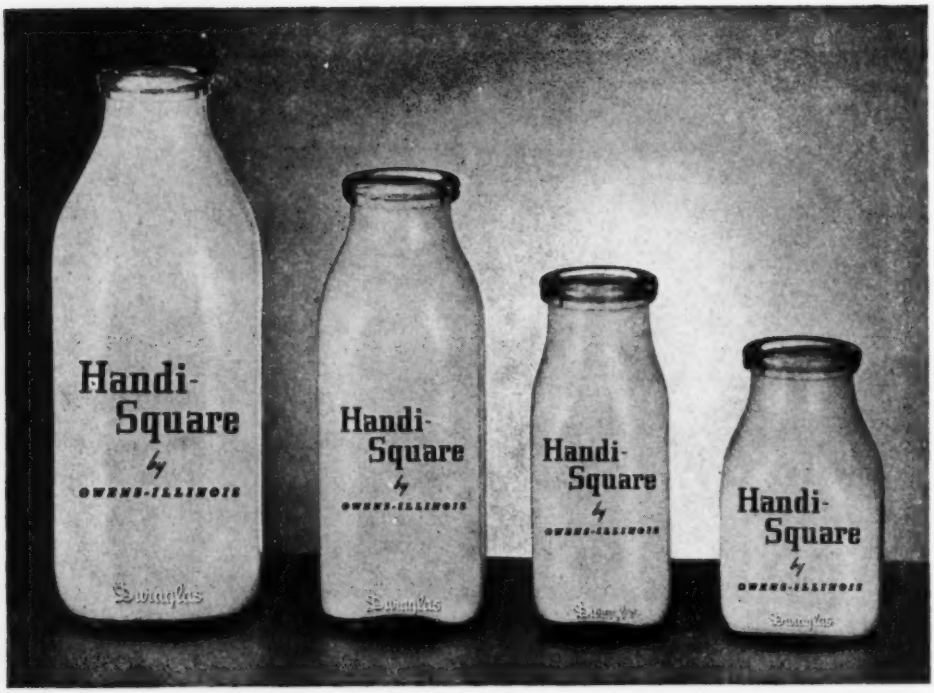

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Dec. 31, 1935	365	324	9079	5.43	492.58	4–7
Dec. 31, 1936	366	325	10666	5.35	570.63	5–7
Dec. 31, 1937	365	332	11696	5.31	621.64	6–7
Dec. 31, 1938	365	329	11294	5.16	582.27	7–7
Dec. 31, 1939	365	320	11094	5.26	583.18	8–7
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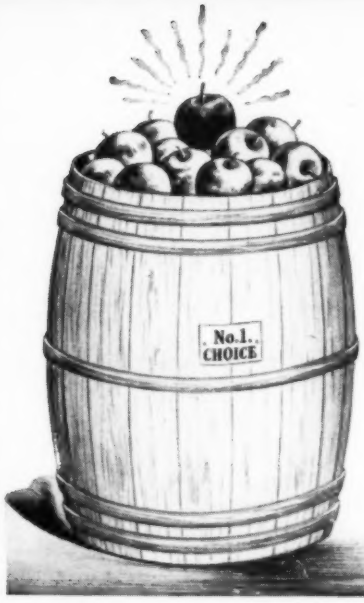

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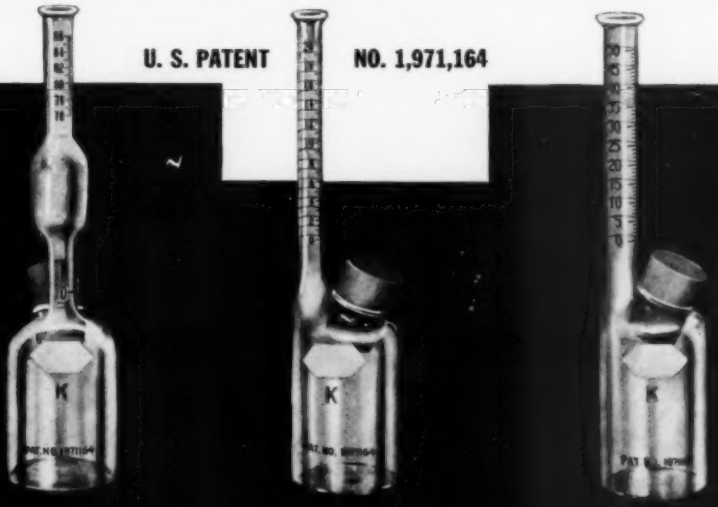

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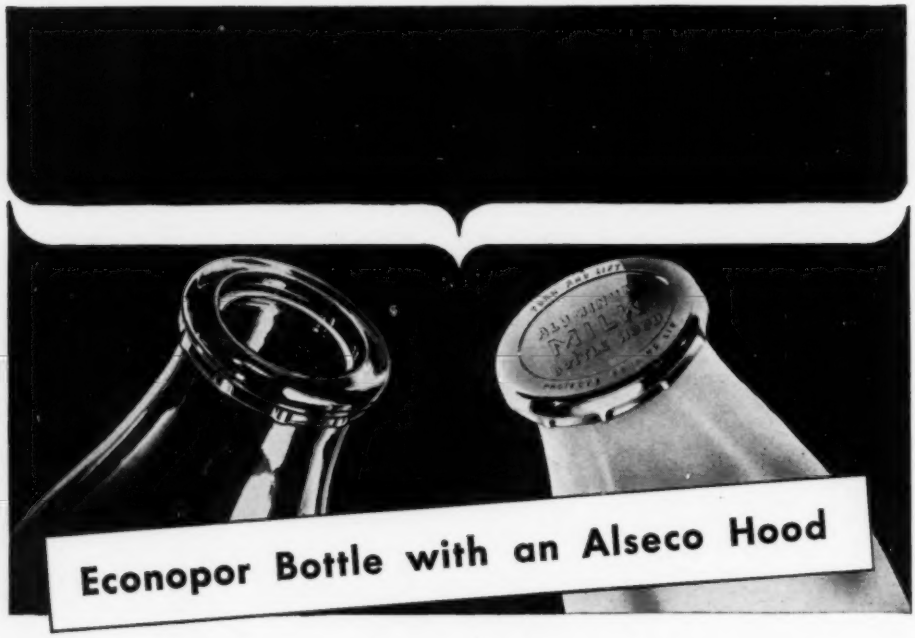

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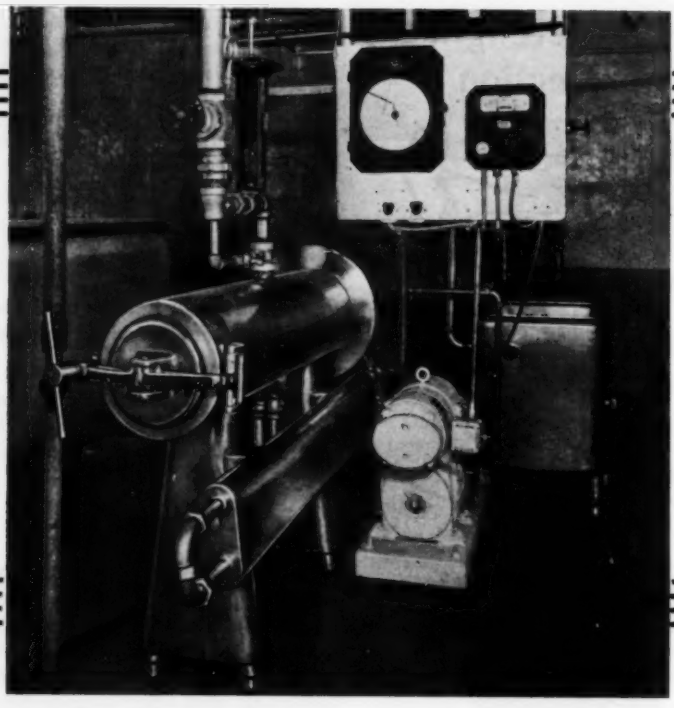

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JOURNAL OF DAIRY SCIENCE

VOLUME XXVIII APRIL, 1945 NUMBER 4

THE INFLUENCE OF BREED, FEED, AND PROCESSING ON THE RIBOFLAVIN CONTENT OF MILK*

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Departments of Dairy Husbandry and Agricultural Chemistry, Idaho Agricultural Experiment Station, Moscow, Idaho

INTRODUCTION

Milk is generally accepted as being the most important source of riboflavin in the human dietary. The riboflavin content of milk is, however, extremely variable as has been demonstrated by various investigators, notably Hand and Sharp (3); Kramer, Dickman, Hildreth, Kunerth, and Riddell (11); Kon (10); and Johnson, Maynard, and Loosli (9). The present study was inaugurated for the purpose of evaluating the influence of various factors on the riboflavin content of milk from representative cows of two breeds throughout a normal lactation period.

PROCEDURE

Samples of milk for the main portion of this study were secured from two Holstein-Friesian and two Jersey cows in the Idaho Agricultural Experiment Station herd. All of the cows were first calf heifers and had freshened within 3 weeks of each other. The cows were milked by machine three times daily and aliquot samples were taken of each milking to make the composite samples on which the riboflavin determinations were made. Samples of milk were taken twice each week, with very few exceptions, for approximately 10 months. Amber glassware was used and all samples were immediately stored in the dark at 40° F. until used for analysis within 24 hours.

The riboflavin content was determined by making an acetone extract as described by Hand (2), and Sharp and Hand (14). A Coleman photofluorometer was used for measuring the fluorescence of the sample before and after the addition of a standard. The blank was determined after reduction with sodium hydrosulfite.

EXPERIMENTAL

Riboflavin content of milk from consecutive milkings. In order to find a

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satisfactory method of securing a daily sample of milk, determinations for riboflavin were made of milk from consecutive milkings of two Holstein and two Jersey cows for a 3-day period. The results are presented in table 1.

The differences in the riboflavin content of individual samples for a single day of any of the cows were within the limits of experimental error. These results are of interest in that they show that, although milk yields for the different milkings during the same day varied considerably, the riboflavin content remained approximately the same for each cow. However, in order to minimize any possible error, and also to minimize labor, it appeared

TABLE 1

Riboflavin content of milk from consecutive milkings for a 3-day period of two Holstein and two Jersey cows

	Holstein No. 64A		Holstein No. 76A		Jersey No. 190X		Jersey No. 196X	
	Milk yield	Riboflavin	Milk yield	Riboflavin	Milk yield	Riboflavin	Milk yield	Riboflavin
	lb./day	*mg./liter*	*lb./day*	*mg./liter*	*lb./day*	*mg./liter*	*lb./day*	*mg./liter*
First day								
Morning	18.9	1.42	16.5	1.41	7.1	3.18	9.8	2.18
Noon	15.8	1.49	16.1	1.49	5.6	3.13	9.6	2.18
Night	16.4	1.47	14.2	1.55	5.4	3.22	9.1	2.28
Second day								
Morning	16.2	1.50	20.1	1.47	7.2	3.17	10.4	2.27
Noon	13.5	1.51	14.6	1.46	5.1	3.13	8.5	2.07
Night	20.1	1.51	16.7	1.46	6.0	3.10	9.2	2.17
Third day								
Morning	18.2	1.45	16.2	1.28	6.6	3.01	10.2	1.94
Noon	11.7	1.49	10.7	1.34	5.2	3.15	8.3	1.98
Night	15.5	1.56	17.1	1.27	5.9	3.10	9.7	1.90

advisable to use 24-hour aliquot composite samples; a procedure followed in this study.

Influence of breed. The average riboflavin content of milk varied with the breed and also varied between different cows of the same breed (figure 1 (part A)). Milk from the Jersey cows was on an average 52 per cent higher in riboflavin than that from the Holstein cows or, stated in another way, the Holstein milk contained about 34 per cent less riboflavin than did Jersey milk. The average value for a 10 months' lactation period for the Holstein milk was 1.44 milligrams per liter, and for the Jersey milk 2.18 milligrams per liter. The range in values for daily samples of milk over this period was 0.88 to 2.23 milligrams per liter for Holstein milk and 1.38 to 3.56 milligrams per liter for Jersey milk. The sharp increase in the average values during December and January is due to the change in feed from dry ration to silage, but the appreciable difference in the riboflavin content of the two breeds is still apparent, as well as the difference between cows of the same breed.

Influence of milk production. The data in figure 1 show that there is an inverse relation between milk yield per day and the riboflavin content of

milk, which supports the observations of Johnson, Maynard, and Loosli (9), and Hand and Sharp (3).

The average riboflavin production in milligrams per day, as calculated from the milk yield and riboflavin content, is plotted in figure 1, part C.

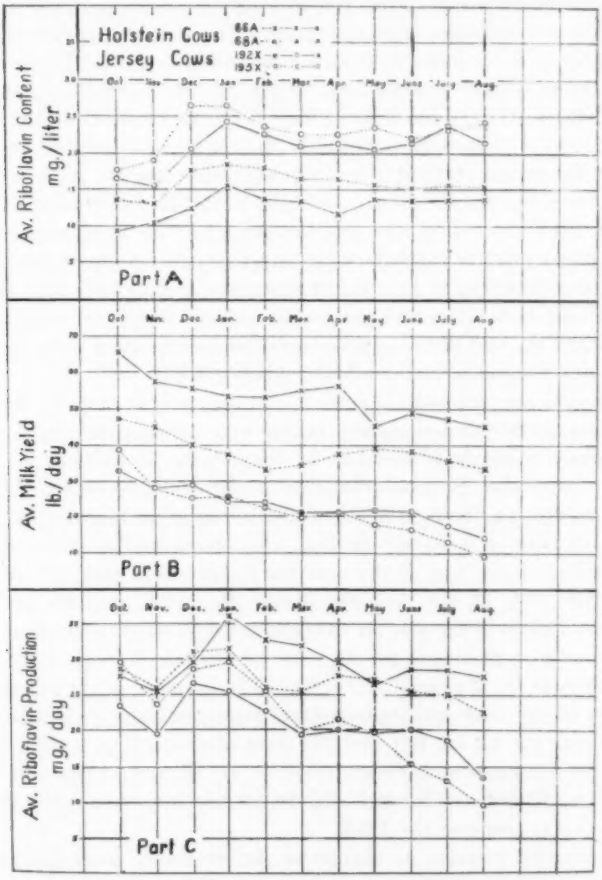

FIG. 1. Average riboflavin content, milk yield, and riboflavin production of four cows representing two breeds.

These results show that the Holstein cows produced an average of 34 per cent more riboflavin per day than did the Jersey cows. The average riboflavin production for the Holstein cows was 28.2 milligrams per day and for the Jersey cows 21.0 milligrams. Although this relationship between the two breeds was not uniform throughout the lactation period, it is apparent that

the Holstein cows did give a higher production of riboflavin than the Jersey cows.

Influence of feed. The cows in this investigation were fed a concentrate mixture of 200 lbs. ground oats, 200 lbs. ground barley, 360 lbs. wheat bran, 200 lbs. linseed oil meal, 50 lbs. pea meal, 20 lbs. bone meal, 10 lbs. iodized salt, and 100 lbs. molasses dried beet pulp. The concentrate mixture was fed individually in accordance with milk production. A good grade of alfalfa hay was fed *ad libitum* as roughage. From the time of calving until December 11, 1943, or 95 days for 66A, 84 days for 68A, 73 days for 192X, and 93 days for 193X, the cows were fed only the concentrate mixture and alfalfa hay. On December 11, 1943, the ration was supplemented daily with 30 lbs. of sunflower silage for the Jersey cows and 36 lbs. for the Holstein cows. On April 29, 1944, the ration was supplemented further by allowing the cows to be on wheat pasture for 2 days for approximately 8 hours each day. On May 15 the cows were put on a mixed pasture of sweet clover and wheat, and then on June 9 both silage and pasture were discontinued for 2 weeks. From June 23 until the end of the experimental period the cows were fed the concentrate–dry roughage, and sunflower silage ration.

The results are presented in table 2. It is apparent that, by supplementing the concentrate–dry roughage ration with substantial amounts of sunflower silage, a material increase in the riboflavin content of the milk resulted. A notable increase averaging 36 per cent occurred within 2 days. After inaugurating the feeding of silage, the average increase in riboflavin per liter of milk in a 10-day period, when compared to a similar period before feeding silage, was 39 per cent for Holstein No. 66A, 39 per cent for Holstein No. 68A, 32 per cent for Jersey No. 192X, and 45 per cent for Jersey No. 193X. This was an average increase of 39 per cent. On the basis of riboflavin produced per day, the percentage increase was 26, 26, 21, and 48 per cent for the respective cows. The increase in the riboflavin concentration of the milk maintained itself comparatively well although at a declining rate for 41, 72, 100, and 131 days after starting to feed sunflower silage. The increases were respectively 85, 47, 36, and 12 per cent for 66A; 30, 24, 7, and 10 per cent for 68A; 72, 42, 13, and 39 per cent for 192X; and 39, 13, 2, and 11 per cent for 193X.

No significant increase or change in the riboflavin concentration of the milk was noted when the cows were put on either wheat pasture or sweet clover and wheat pasture. During a 13-day period after discontinuing the feeding of sunflower silage, there appeared to be a slight tendency toward a decrease in the riboflavin concentration in the milk. However, although this trend was indicated in the milk from each cow, the overall decrease in riboflavin concentration was no greater than several times observed between samples of two consecutive sampling periods. It is possible that the experimental period of 13 days, during which the cows were without silage and pas-

TABLE 2

Influence of feed on the riboflavin content of milk

Date	66A			68A			192X			193X		
	Milk, lb.	Mg. per liter	Mg. per day	Milk, lb.	Mg. per liter	Mg. per day	Milk, lb.	Mg. per liter	Mg. per day	Milk, lb.	Mg. per liter	Mg. per day
Dec. 3	61.2	0.88	23.9	45.0	1.48	29.3	29.0	1.78	22.7	26.0	2.15	24.9
Dec. 7	59.9	0.92	24.2	40.8	1.40	25.1	31.9	1.46	20.5	24.5	2.11	22.7
Dec. 10	55.7	1.07	26.2	39.0	1.44	24.7	31.3	1.87	25.8	19.2	2.18	18.4
Dec. 11	Started to feed sunflower silage											
Dec. 14	53.5	1.50	35.3	38.4	1.95	29.9	26.3	2.35	27.2	24.1	3.19	33.8
Dec. 17	56.2	1.24	30.1	39.4	2.23	38.6	26.8	2.20	25.9	25.4	3.56	39.8
Dec. 21	50.9	1.27	28.4	39.6	1.81	31.5	30.9	2.23	30.3	26.3	2.63	30.4
Jan. 21	51.7	1.78	40.5	35.8	1.97	31.0	24.3	2.93	31.3	17.4	2.99	26.6
Feb. 21	49.1	1.41	30.4	31.7	1.78	24.8	19.6	2.42	20.9	20.6	2.43	22.0
Mar. 20	57.1	1.31	32.9	36.7	1.57	25.3	22.1	1.92	18.7	18.1	2.20	17.5
Apr. 20	49.7	1.07	23.4	36.1	1.59	25.2	19.1	2.33	19.6	22.9	2.40	24.2
Apr. 23	54.7	1.17	28.1	38.0	1.58	26.4	22.7	2.45	24.5	23.0	2.25	22.7
Apr. 27	58.8	1.23	31.8	40.0	1.72	30.3	20.8	2.16	19.7	23.3	2.34	24.0
Apr. 29	Put on wheat pasture for 2 days											
May 1	55.7	1.28	31.4	40.1	1.53	27.0	20.8	1.87	17.1	19.1	2.24	18.8
May 4	54.3	1.49	35.6	39.0	1.64	28.1	20.1	2.12	18.7	18.6	2.25	18.4
May 8	54.4	1.46	34.9	39.5	1.53	26.5	22.0	1.83	17.7	15.7	2.88	19.9
May 11	21.1	1.83	17.0	40.3	1.49	26.4	22.3	1.98	19.4	16.4	2.35	16.9
May 15	Put on sweet clover and wheat pasture											
May 18		Mastitis		39.3	1.54	26.6	22.4	2.19	21.6	19.1	1.93	16.2
May 22		Mastitis		36.0	1.52	24.1	20.9	2.05	18.8	20.0	2.48	21.8
May 25	46.9	1.43	29.5	39.4	1.64	28.4	22.7	2.24	22.4	20.4	2.45	22.0
June 8	48.7	1.32	28.3	38.8	1.63	27.8	21.7	2.18	20.8	19.2	2.30	19.4
June 9	Sunflower silage and pasture discontinued											
June 12	51.0	1.23	27.6	38.8	1.53	26.1	22.9	1.89	19.0	14.5	2.17	13.8
June 15	47.3	1.36	28.3	38.4	1.63	27.5	22.7	2.14	21.4	15.9	2.37	16.6
June 19	50.0	1.27	27.9	38.6	1.41	23.9	20.6	1.89	17.1	12.6	1.84	10.2
June 22	51.8	1.24	28.2	37.1	1.55	25.3	21.3	1.84	17.2	11.6	1.95	9.9

ture, was not long enough to dissipate the influence of these feeds. It has been established by Bechdel, Honeywell, Dutcher, and Knutsen (1), Hunt, Burroughs, Bethke, Schalk, and Gerlaugh (8), and McElroy and Goss (13) that riboflavin can be synthesized in the rumen, which might also have been a factor influencing the results of the short experimental period.

Influence of stage of lactation, season, and pregnancy. During a 310-day period there was no significant influence of any stage of lactation or season on the riboflavin content of the milk. All changes in riboflavin concentration were satisfactorily related to other factors or influences.

No discernible influence on the riboflavin content of the milk was exerted by pregnancy. Holstein cow No. 66A was pregnant for 100 days of the experimental period, Holstein cow No. 68A for 119 days, Jersey cow No. 192X for 225 days, and Jersey cow No. 193X for 111 days. Reference to

TABLE 3

Influence of oestrus on the riboflavin content of milk

	Holstein 66A	Holstein 68A	Jersey 192X	Jersey 193X
Number oestrus periods	4	2	2	3
Average milk yield preceding day (lb.)	53.60	35.70	30.60	22.20
Average milk yield oestrus day (lb.)	54.20	34.20	29.70	23.20
Average milk yield following day (lb.)	54.30	38.20	27.50	24.20
Average mg./l. (riboflavin) preceding day	1.32	1.66	1.74	2.52
Average mg./l. (riboflavin) oestrus day	1.28	1.87	2.04	2.46
Average mg./l. (riboflavin) following day	1.43	1.59	2.44	2.35
Average mg. (riboflavin) preceding day	31.10	25.70	23.30	24.70
Average mg. (riboflavin) oestrus day	30.50	28.30	26.60	25.30
Average mg. (riboflavin) following day	34.10	26.70	29.60	25.40

figure 1 (part A) shows no change which could be correlated with conception or advance in gestation.

Influence of oestrus. According to the data obtained, oestrus has no apparent effect on either the riboflavin content of milk or the total amount of riboflavin produced per day. Data presented in table 3 show no consistent or positive indication of oestrus influencing the riboflavin content of milk.

Riboflavin content of colostrum milk. Kramer, Gardner, Kunerth, and Riddell (12) have indicated that colostrum milk is higher in riboflavin than later milk. Table 4 shows the riboflavin concentration of colostrum milk drawn within 10 hours after calving as compared with milk from the same cows for 8 consecutive days after calving and then again on the 20th day. The Holstein colostrum milk, sampled within 10 hours after calving, averaged 5.89 milligrams of riboflavin per liter and the Jersey milk 5.42 milligrams.

Results show that colostrum milk rapidly lost its high concentration of riboflavin (30 per cent within 24 hours). If milk produced on the 20th day

TABLE 4

Riboflavin content of colostrum milk and milk samples for some days following calving

Days after calving milk was sampled*	Holstein cows		Jersey cows	
	57A	11A	180X	103A
	mg./l.	*mg./l.*	*mg./l.*	*mg./l.*
0	6.38	5.41	5.01	5.83
1	4.34	3.46	3.68	4.15
2	2.68	2.97	2.81	2.77
3	2.19	2.91	2.75	2.79
4	1.89	2.23	.....	2.52
5	1.95	2.07	2.70	2.41
6	1.82	2.13	2.53	2.33
7	1.81	1.94	2.37	2.33
8	1.83	2.06	2.22	2.34
20	1.77	1.89	1.97	2.38

* First sample taken within 10 hours after calving.

after calving is considered as normal, it is evident that, in so far as riboflavin content is concerned, milk is normal in 6 to 8 days after calving. The riboflavin content of the colostrum milk (sample taken within 10 hours of calving) in comparison with normal milk was 3.6 times higher for Holstein cow No. 57A, 2.8 for Holstein cow No. 11A, 2.5 for Jersey cow No. 180X, and 2.4 for Jersey cow No. 103A. These results indicate that colstrum milk is from two and one-half to three and one-half times as high in riboflavin as later or normal milk.

Influence of processing. Milk was pasteurized on three separate days by heating to 143° F. and holding for 30 minutes. Some of the same milk was homogenized at 1500 lbs. pressure by a CP Multi-Flo homogenizer. Samples of both pasteurized and pasteurized-homogenized milk were stored at 40° F. for 24 hours in a dark refrigerator.

Results presented in table 5 definitely show that neither pasteurization nor homogenization decreased the riboflavin concentration of milk. In addition, the data show that both pasteurized and homogenized milk can be stored for at least 24 hours at 40° F. in a dark refrigerator without any loss of riboflavin.

TABLE 5

Effect of pasteurization and homogenization on the riboflavin content of milk on three different days

Treatment	Riboflavin content, mg./l.		
Raw milk, just before pasteurization	1.73	1.81	1.77
Pasteurized—unhomogenized. No storage	1.72	1.80	1.81
Same, after 24 hours storage at 40° F.	1.71	1.83	1.85
Pasteurized—homogenized. No storage	1.74	1.79	1.81
Same, after 24 hours storage at 40° F.	1.71	1.82	1.83

DISCUSSION OF RESULTS

The influence of breed (Holstein-Friesian and Jersey) on the riboflavin content of milk was clearly demonstrated by this study. The results covering a normal lactation period (approximately 10 months) are in general agreement with the work of Whitnah, Kunerth, and Kramer (17), Kramer, Dickman, Hildreth, Kunerth, and Riddell (11), and Hand and Sharp (3). Although the Holstein milk contained an average of 34 per cent less riboflavin than did the Jersey milk, the total daily yield of riboflavin was 34 per cent more for the Holstein cows. Differences between cows of the same breed were also observed.

Virtanen and Holmberg (16), Whitnah, Kunerth, and Kramer (17), Kramer, Dickman, Hildreth, Kunerth, and Riddell (11), Hand and Sharp (3), and Johnson, Maynard, and Loosli (9) have found that diet influences the riboflavin content of milk. The present study showed that, when a concentrate–dry roughage ration was supplemented with sunflower silage, the riboflavin content of the milk was increased approximately 36 per cent within 2 days. After the initial increase of riboflavin, as the result of feeding sunflower silage, there was a decrease in riboflavin content but the concentration remained higher than before silage was fed.

Results here reported are of special interest in indicating that, on a ration including substantial amounts of sunflower silage, the riboflavin concentration of milk is comparatively constant after the initial effect of the silage has been lost and is higher than milk produced on a concentrate–dry roughage ration. This supports the work of Virtanen and Holmberg (16) using A.I.V. silage, and the observations of Holmes and Holmes (5).

Although colostrum milk is a good source of riboflavin, it is of almost equal significance that colostrum rapidly loses its high concentration of riboflavin. With this in mind the usual recommendation of giving the calf an early advantage in life by feeding the first milk assumes added importance.

Results showing that the pasteurization process causes no loss of riboflavin are in agreement with those of Houston, Kon, and Thompson (7), Holmes, Jones, Wertz, and Kuzmeski (6), and Holmes (4). The study further established that homogenization of pasteurized milk does not decrease the riboflavin content nor does storage in the dark for 24 hours at 40° F. in a refrigerator. Although processing does not decrease the riboflavin content of milk, Stamberg and Theophilus (15) have clearly demonstrated the detrimental influence of sunlight on milk in clear glass bottles.

SUMMARY

Experiments with two Holstein-Friesian and two Jersey cows over a normal lactation period of approximately 10 months (samples taken twice weekly) showed:

1. The milk of two Holstein cows used in this experiment contained, on an average, approximately 34 per cent less riboflavin than that of two Jersey cows.

2. The Holstein cows, because of higher milk production, produced on an average approximately 34 per cent more riboflavin per day than the Jersey cows.

3. There was an inverse relation between milk yield per day and the riboflavin content.

4. The riboflavin content of morning, noon, and night milk from the same cow was practically constant although the milk yields varied widely.

5. Supplementing a concentrate–dry roughage ration with substantial amounts of sunflower silage increased the riboflavin content of milk 36 per cent (within 2 days), and the riboflavin content level was comparatively high for the remaining portion of lactation while the silage was fed.

6. Supplementing a concentrate–dry roughage sunflower–silage ration with wheat pasture or sweet clover and wheat pasture caused no significant change in the riboflavin content of milk.

7. Stage of lactation, season, pregnancy, and oestrus have no discernible influence on the riboflavin content of milk.

8. Colostrum milk contains about three times as much riboflavin as normal milk but loses approximately 30 per cent of its riboflavin concentration within 24 hours after parturition of the cow.

9. Neither pasteurization, homogenization, or storage for 24 hours at 40° F. in a dark refrigerator decreased the riboflavin content of milk.

REFERENCES

(1) Bechdel, S. J., Honeywell, H. E., Dutcher, R. A., and Knutsen, M. H. Synthesis of Vitamin B in the Rumen of the Cow. Jour. Biol. Chem., 80: 231. 1928.

(2) Hand, David B. Determination of Riboflavin in Milk by Photoelectric Fluorescence Measurements. Indus. and Engin. Chem., Analyt. Ed., 11: 306. 1939.

(3) Hand, D. B., and Sharp, P. F. The Riboflavin Content of Cow's Milk. Jour. Dairy Sci., 22: 779. 1939.

(4) Holmes, A. D. Effect of Pasteurization on the Riboflavin Content of Milk. Amer. Dietet. Assoc. Jour., 20: 226. 1944.

(5) Holmes, A. D., and Holmes, J. O. Uniformity of Riboflavin Content of Milk Produced under Standardized Conditions. Amer. Jour. Dis. Children, 66: 607. 1943.

(6) Holmes, A. D., Jones, C. P., Wertz, A. W., and Kuzmeski, J. W. The Ratio of Ascorbic Acid, Riboflavin and Thiamine in Raw and Pasteurized Milk. Jour. Nutr., 26: 337. 1943.

(7) Houston, J., Kon, K., and Thompson, S. Y. The Effect of Commercial Pasteurization and Sterilization on the Vitamin B and Riboflavin Content of Milk as Measured by Chemical Methods. Jour. Dairy Res., 11: 67. 1940.

(8) Hunt, C. H., Burroughs, E. W., Bethke, R. M., Schalk, A. F., and Gerlaugh, P. Further Studies on Riboflavin and Thiamine in the Rumen Content of Cattle. II. Jour. Nutr., 25: 207. 1943.

(9) JOHNSON, PAUL, MAYNARD, L. A., AND LOOSLI, J. K. The Riboflavin Content of Milk as Influenced by Diet. JOUR. DAIRY SCI., 24: 57. 1941.

(10) KON, S. K. The Nutritional Value of Milk and Milk Products. Jour. Dairy Res., 11: 196. 1940.

(11) KRAMER, M. M., DICKMAN, R. M., HILDRETH, M. D., KUNERTH, B. L., AND RIDDELL, W. H. The Riboflavin Value of Milk. JOUR. DAIRY SCI., 22: 753. 1939.

(12) KRAMER, M. M., GARDNER, I., KUNERTH, B. L., AND RIDDELL, W. H. Vitamin G (Riboflavin) Content of Colostrum and Milk of Cows Determined Biologically. Jour. Agr. Res., 56: 233. 1938.

(13) MCELROY, W. W., AND GOSS, W. A Quantitative Study of Vitamins in the Rumen Contents of Sheep and Cows Fed Vitamin-Low Diets. I. Riboflavin and Vitamin K. Jour. Nutr., 20: 527. 1940.

(14) SHARP, P. F., AND HAND, D. B. Riboflavin, Vitamin C and Flavor of Dairy Products. Proc., 1st Food Conf. Inst. Food Technol., 1: 139. 1940.

(15) STAMBERG, O. E., AND THEOPHILUS, D. R. Loss of Riboflavin in Milk Due to Sunlight. Milk Dealer, 33: 32. 1944.

(16) VIRTANEN, A. V., AND HOLMBERG, T. Variations of Vitamin B_2 in Milk. Suomen Kemistilehti, 11B: 1–2. 1938. Chem. Abs., 32: 3037. 1938.

(17) WHITNAH, C. H., KUNERTH, B. L., AND KRAMER, M. M. Riboflavin Content of Milk Collected in Different Months and Correlated with Other Constituents of the Milk. JOUR. DAIRY SCI., 21: 593. 1938.

PHOTOLYSIS OF RIBOFLAVIN IN MILK*

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Riboflavin is known to be quite thermostable; but this important vitamin readily undergoes photolysis. The significance of this photolability in foods was shown to some extent by Williams and Cheldelin (7), who also observed that riboflavin solutions were more stable at pH 3.0 than at pH 6.5 and that higher temperatures increased the riboflavin destruction by light. O'Malley and Sievert (3) made the interesting observation that solutions of reduced riboflavin were quite photostable.

Williams and Cheldelin (7) observed that milk boiled in a light room lost considerable amounts of riboflavin as compared to a very slight loss when the milk was boiled in the dark. A brief note was published by Peterson, Haig, and Shaw (4) showing that pint bottles of milk exposed to sunlight for one hour lost from 30 to 54 per cent riboflavin and that the loss was greater upon longer light exposure. About the same time a fairly extensive study on this subject was reported by Stamberg and Theophilus (6), who used quart-size bottles throughout and included a study of raw, pasteurized, and homogenized milk packaged in clear and brown glass bottles and paper containers, exposed to direct sunlight and simultaneous shade. Although this work was conducted during the winter months at near freezing temperatures, they found that as much as 80 per cent of the riboflavin of milk in clear bottles was destroyed after 6 hours' exposure to direct sunlight, but less than 10 per cent loss from the same milk in paper containers and brown glass bottles. The loss of riboflavin was also very much less in shade as compared to direct sunlight. Stamberg and Theophilus also showed that Jersey milk of high riboflavin content lost more riboflavin than Holstein milk of lower riboflavin content when exposed to sunlight at the same time. A note by Ziegler (8) confirms the results that riboflavin, as determined by microbioassay, is readily destroyed in milk exposed to sunlight. The other workers cited used the photofluorometric method.

The work in this report includes a study of riboflavin loss of milk exposed to sunlight during the conditions of the summer months and effects of different types of containers, temperature, storage, and cooking.

EXPERIMENTAL

Analytical method. The riboflavin determinations were made by making an acetone extract as described and used by Hand (2) and Sharp and Hand

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(5) except that 10 ml. acetone and 5 ml. milk were used. The fluorescence of the diluted extract was determined before and after the addition of a standard and after reduction with sodium hydrosulfite for obtaining the blank value. A Coleman photofluorometer with the appropriate filter was used.

Effects of milk type, sun and shade. Milk from a mixed herd of Jersey and Holstein cows was used. From the same milk, raw, pasteurized, and pasteurized-homogenized samples were bottled in clear quart bottles. The milk was stored at 42° F. until taken outside for exposure to direct sunlight or in good shade. The time was from 8:30 A.M. to 10:30 A.M. on a summer day with a few scattered clouds. The results are shown in table 1. The

TABLE 1

Loss of riboflavin in milk exposed in clear quart glass bottles to direct sunlight and in simultaneous good shade

Type of milk	Light exposure		Temperature of milk after exposure	Riboflavin content	Riboflavin loss
	Type	Hours			
			°F.	mg./l.	%
Raw	.....	0	.....	1.62	.....
"	Sun	1	62–64	1.29	20.4
"	Sun	2	64–66	0.97	40.1
"	Shade	1	55–57	1.52	6.2
"	Shade	2	57–59	1.51	6.8
Pasteurized	.....	0	.....	1.64	.....
"	Sun	1	62–64	1.29	21.3
"	Sun	2	64–66	1.06	35.4
"	Shade	1	55–57	1.59	3.0
"	Shade	2	57–59	1.58	3.7
Pasteurized-homogenized	.....	0	.....	1.63	.....
"	Sun	1	62–64	1.41	13.5
"	Sun	2	64–66	1.18	27.6
"	Shade	1	55–57	1.62	0.6
"	Shade	2	57–59	1.58	3.1

loss of riboflavin in direct sunlight after 2 hours was from 27 to 40 per cent compared to only 3.1 to 6.8 per cent loss in the shade. Homogenized milk lost the least in 2 hours, or 27.6 per cent, as compared to 35.4 per cent for pasteurized milk and 40.1 per cent for raw milk. Thus the opacity of the homogenized milk gave some protection, but the loss was still appreciable.

Effect of light and subsequent dark storage. Pasteurized-homogenized milk in clear quart glass bottles was used in this experiment. The bottles were exposed to direct sunlight for 1, 2, or 3 hours and then stored in the dark in a refrigerator at 42° F. up to 20 hours. The loss of riboflavin due to direct sunlight and subsequent dark as shown by the data in table 2 indicates that, after the milk has been removed from the light source, there is no further destruction of the riboflavin. This is interesting in that there is apparently no residual effect on milk exposed to light for a brief period.

Fuhr, Dornbush, and Peterson (1) found that irradiation of market milk with ultraviolet light to produce 400 units of vitamin D per quart did not reduce the riboflavin content.

Effects of temperature. The effect of temperature on the riboflavin loss due to light was studied, using raw and pasteurized-homogenized milk. A water thermostat of glass was used, into which a long glass tube was inserted, containing in each instance 50 ml. of milk. A General Electric sun lamp with an F1 bulb placed 14 inches from the thermostat was used as a light source. All conditions were maintained constant except for the temperature of the thermostat, and a thermometer in the milk was used to check the milk temperatures. For these experiments, temperatures of 96, 60, 30

TABLE 2

Effect of exposure of pasteurized-homogenized milk in clear glass bottles to direct sunlight and subsequent storage in a refrigerator in the dark at 42° F. on the riboflavin content

Exposure		Riboflavin content
Direct sunlight	Subsequent dark storage	
hours	*hours*	*mg./l.*
0	0	1.78
1	0	1.47
1	5	1.47
1	20	1.50
2	0	1.15
2	4	1.14
2	20	1.18
3	0	0.90
3	3	0.93
3	20	0.90

and 3° C. with 30 and 60 minutes' exposure to light were studied. Employing a similar thermostat standing in an open window, exposures to direct sunlight were also made of the same milk lots.

The results of these experiments are shown in table 3. The data indicate that temperature is an important factor. Raw milk in each instance lost more riboflavin than the pasteurized-homogenized milk of the same milk lot. The light intensity of the lamp was not as great as that of direct sunlight at noon, but served to show the effect of temperature. Thus, milk which is kept cold will lose considerably less riboflavin when exposed to light than when kept at higher temperatures.

Effect of containers. The protective action of brown glass and paper containers against riboflavin destruction was shown by Stamberg and Theophilus (6), but a more complete study was made to include four types of paper quart containers, as well as clear glass bottles, with the usual amount of printing and brown quart (beer) bottles. Paper containers No. 1 and

TABLE 3

Effect of temperature on the loss of riboflavin in milk

Type of milk	Temperature of milk during light exposure	Light exposure		Riboflavin	
		Type	Minutes	Content	Loss
	°C.			mg./l.	%
Raw	.....	.....	0	1.56	.....
"	96	Lamp	30	1.05	32.7
"	60	"	30	1.36	12.8
"	30	"	30	1.39	8.9
"	3	"	30	1.47	5.8
Raw	.....	.....	0	1.56	.....
"	96	Lamp	60	0.72	53.8
"	60	"	60	1.15	26.3
"	30	"	60	1.34	14.1
"	3	"	60	1.45	7.1
Pasteurized-homogenized	.....	.....	0	1.57	.....
"	96	Lamp	30	1.23	21.7
"	60	"	30	1.45	7.6
"	30	"	30	1.53	2.5
"	3	"	30	1.55	1.3
Pasteurized-homogenized	.....	.....	0	1.57	.....
"	96	Lamp	60	0.86	45.2
"	60	"	60	1.29	17.8
"	30	"	60	1.49	5.6
"	3	"	60	1.54	1.9
Raw	28	Sun	30	0.68	56.4
"	28	Sun	60	0.34	78.2
Pasteurized-homogenized	28	Sun	30	0.90	42.7
"	28	Sun	60	0.55	65.0

No. 2 were of the conical type and No. 3 and No. 4 were rectangular. Pasteurized-homogenized milk was used with an original riboflavin content of 1.78 mg./l. The results are shown in table 4. Brown glass and paper con-

TABLE 4

Loss of riboflavin of pasteurized-homogenized milk exposed to direct sunlight in clear and brown glass bottles and four brands of paper quart containers. The original riboflavin content of the milk was 1.78 mg./l.

Length of light exposure	Temperature of milk after exposure	Containers					
		Clear glass	Brown glass	Paper No. 1	Paper No. 2	Paper No. 3	Paper No. 4
		Riboflavin loss, per cent					
hours	°F.						
½	48–50	9.0	0.6	1.7	0.6	2.2	3.4
1	62–64	17.4	1.7	2.2	1.7	3.9	5.1
2	67–69	35.4	4.5	3.2	2.2	5.6	7.6
3	74–76	49.4	5.1	5.1	3.4	6.5	10.7
4	80–82	62.9	5.6	5.2	4.5	3.9	14.6
6	85–87	77.5	5.6	5.3	5.6	6.6	16.9

TABLE 5

Loss of riboflavin in milk stored in clear quart bottles in the showcases of two stores

Storage time	Store A		Store B		Check, in dark at 42° F.	
	Mg./l.	Loss, per cent	Mg./l.	Loss, per cent	Mg./l.	Loss, per cent
hours						
0	1.64	.....	1.64	.....	1.64	.....
24	1.58	3.7	1.65	0	1.65	0
48	1.54	7.3	1.61	0.2	1.65	0

tainers gave a very good protection against riboflavin loss as compared to the clear glass bottles. Only one paper container (No. 4) proved to be slightly less efficient in this respect.

Loss in showcases. Possible loss of riboflavin in store showcases was studied in two places. Store A has a glass showcase about 10 feet from a large window facing east. Store B has a large showcase about 75 feet from windows facing north, and most of the light is from six 30-watt fluorescent lamps in the case. Pasteurized milk in clear glass quart bottles was allowed to stand in these showcases up to 48 hours. A set of check bottles were stored in the dark at 42° F. The days of these experiments were partly cloudy. The results are shown in table 5. Very little riboflavin was lost by storage in store B, while a significant amount was lost in store A after 48 hours. On a very clear day it is expected that the loss would be somewhat higher in store A. There was no loss of riboflavin upon storage in the dark at 42° F. for 48 hours.

Loss on cooking. The loss of riboflavin during cooking of milk was studied. One pan was made of glass and one of aluminum, with lids of the same material. Each pan was of 1½ quart size and 5 inches in diameter. The amount of milk used each time was 250 ml. About 5 minutes was required to bring the milk to boil. Pan and milk were weighed before heating, and loss in moisture during heating was compensated for by distilled water. There was brilliant sunshine outside. The cooking took place about 7 feet

TABLE 6

Effect on the riboflavin content of heating milk in aluminum and glass pans

Pan used	Total heating time, minutes	Riboflavin	
		Mg./l.	Loss, per cent
(Original milk)	0	1.76	.....
Glass, covered	15	1.61	8.5
" "	30	1.57	10.8
Aluminum, no cover	15	1.59	9.7
" " "	30	1.55	11.9
Aluminum, covered	30	1.74	1.1

from large windows, but there was no direct sunlight on the pans. After the milk came to a boil, it was allowed to simmer. The results are shown in table 6 for 15 and 30 minutes of total heating time. There was a significant loss of riboflavin when milk was allowed to simmer in the glass pan with lid, or aluminum pan without lid; while in an aluminum pan with lid excluding practically all light there was no appreciable loss. Thus, even during the cooking of milk some riboflavin can be lost if exposed to the light conditions of a well-lighted kitchen, and the data in table 3 show that at near boiling temperatures photolysis of riboflavin is increased.

DISCUSSION AND SUMMARY

Riboflavin is one of the important vitamins and probably is a limiting factor in the average diet. Since milk is probably the most regularly relied upon source of riboflavin for children as well as most adults, precautions should be taken to prevent photolysis of riboflavin before milk is consumed.

The results of this study show that direct sunlight exposure of milk is very detrimental to the riboflavin content. It is a common practice to deliver milk bottles on doorsteps where they stand fully exposed for long periods. A shady place or lightproof box or cabinet should be provided unless protective types of containers are adopted.

The results of this work show that as much as 40 per cent of the riboflavin in milk was destroyed after two hours' exposure to direct sunlight in clear quart bottles even though the milk was quite cool. Good shade, brown glass bottles or paper containers gave very good protection. One type paper container was less efficient in this respect than three other types.

After milk was exposed to sunlight and subsequently stored in the dark in a refrigerator, there was no further loss of riboflavin for the 20 hours investigated.

Temperature is an important factor, and the photolysis of riboflavin at near freezing temperature was low as compared to near boiling temperature. The photolysis of riboflavin in raw milk was generally greater than in pasteurized milk and least in homogenized milk.

Milk kept in a store showcase close to a window lost some riboflavin, but there was practically no loss in milk stored in a showcase where most of the light came from low intensity electric lights.

As much as 11–12 per cent of the riboflavin was lost due to daylight when milk was allowed to simmer for 30 minutes in covered glass pan or uncovered aluminum pan in a bright room, but there was practically no loss when a covered aluminum pan was used.

REFERENCES

(1) FUHR, I., DORNBUSH, A. C., AND PETERSON, W. H. The Effect of Ultraviolet Irradiation on the Vitamin A, Carotene, and Riboflavin Content of Milk. JOUR. DAIRY SCI., 26: 643–646. 1943.

(2) HAND, DAVID B. Determination of Riboflavin in Milk by Photoelectric Fluorescent Measurements. Indus. and Engin. Chem., Analyt. Ed., **11**: 306. 1939.

(3) O'MALLEY, C. M., AND SIEVERT, C. W. Effect of Light on Riboflavin Solutions. Indus. and Engin. Chem., **34**: 1117–1118. 1942.

(4) PETERSON, W. J., HAIG, F. M., AND SHAW, A. O. Destruction of Riboflavin in Milk by Sunlight. Amer. Chem. Soc. Jour., **66**: 662–663. 1944.

(5) SHARP, PAUL F., AND HAND, DAVID B. Riboflavin, Vitamin C and Flavor of Dairy Products. Proc., 1st Food Conf. Inst. Food Technol., **1**: 139–144. 1940.

(6) STAMBERG, OLOF E., AND THEOPHILUS, D. R. Loss of Riboflavin in Milk Due to Sunlight. Milk Dealer, **33**, No. 8: 32, 42. May, 1944.

(7) WILLIAMS, R. R., AND CHELDELIN, V. H. Destruction of Riboflavin by Light. Sci., **96**: 22–23. 1942.

(8) ZIEGLER, JOHN A. Photochemical Destruction of Vitamin B_2 in Milk. Amer. Chem. Soc. Jour., **66**: 1039–1040. 1944.

THE ATTRACTION BETWEEN THE FAT GLOBULES AND THE LEUCOCYTES OF MILK*

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The problem of sedimentation in homogenized milk, which continues to confront the processor of such milk, made it imperative to study further the relationship between the leucocytes and the milk fat globules.

The presence of excessive numbers of leucocytes is generally regarded as the most important single contributing factor to sedimentation in homogenized milk. Babcock (1) showed the migration of the leucocytes with the rising fat globules in nonhomogenized pasteurized milk and their settling out in the homogenized product. Similar results have been obtained by other investigators. Recently Trout and associates (2) demonstrated that over 95 per cent of the leucocytes were carried into the cream layer of nonhomogenized milk. The upward movement of the leucocytes has been attributed to the sweeping action of the rising fat globules. In the absence of creaming, as in properly homogenized milk, the leucocytes tend to settle and form a deposit of sediment upon prolonged storage of the milk.

Since data had shown the migration of leucocytes with the rising fat globules in nonhomogenized milk, it seemed of interest to find if, and to what extent, the normal fat globules would descend with the leucocytes if sufficiently large numbers of the leucocytes were present in the milk. A knowledge of the attraction between leucocytes and fat globules might thus furnish a clue to a method of homogenizing milk without the problem of sedimentation.

EXPERIMENTAL

Increasing amounts of washed leucocytes were added to pint bottles of fresh, pasteurized milk, after which each bottle was shaken twenty-five times and placed in storage at 40° F. for sufficient length of time to allow for the natural undisturbed migration of both the fat globules and the leucocytes. The washed leucocytes were secured by repeated washing of fresh separator slime in physiological salt solutions, centrifuging and decanting. After sufficient storage the bottles were examined for cream volume, sediment formation, distribution of leucocytes and the percentages of fat in the upper, middle and lower thirds of each pint bottle.

The leucocyte counts were made by using the improved Neubauer counting chamber and Toisson's fluid as a stain. Fat determinations were made by the regular Babcock method. For photographic purposes 10 ml. of a saturated solution of Sudan III was added to each pint of milk.

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RESULTS

The addition of washed leucocytes to nonhomogenized milk either enhanced or depressed the creaming ability of milk, depending upon the amounts added. The depth of the cream layer increased with added increments of washed leucocytes until finally the "carrying capacity" of the fat was reached, at which stage both an upper and a lower cream layer were formed (table 1, and figure 3). No lower "cream" layer was observed upon the addition of up to 12.5 gm. of washed leucocytes per pint of milk. However, the upper cream volume was increased noticeably by small increments of washed leucocytes as shown in table 1. When 12.5 gm. of washed leucocytes per pint of milk was added, the upper third of the milk contained over one hundred million leucocytes per ml., which was 95.8 per cent of the total

TABLE 1

The influence of added leucocytes on the creaming ability of milk

Leucocytes added (gm./pint)	Volume in ml. per pint of the		
	Upper cream layer	Skim milk layer	Lower cream layer
0.0	63	412	0
2.5	58	417	0
5.0	66	409	0
7.5	70	405	0
10.0	74	401	0
12.5	77	398	0
15.0	45	390	40
20.0	40	390	45
25.0	35	385	55
30.0	30	390	60
45.0	23	365	87
60.0	15	360	100

number present in the milk (table 2). These were associated with 92.8 per cent of the total fat present.

Upon the further addition of 2.5 gm. of the leucocytes the "breaking point" of the cream line, or the "carrying capacity" of the fat, was reached, whereupon the leucocyte count in the upper layer dropped to approximately thirty-five million per ml. and continued to decrease in numbers upon the further addition of increasing amounts of leucocytes, as shown in column 3, table 2, and in figure 1. The divided cream volume was associated with a marked decrease of fat in the upper layer.

The data in figure 3 show clearly the effect of the leucocytes in general upon the distribution of fat in the milk. Data showing the migration of the fat with the leucocytes towards the bottom of the bottle are presented in table 3 and figure 2. The correlation between the number of leucocytes and the percentage of fat can be observed by comparing the data of tables 2 and 3, and of figure 2. It is of interest to note that the fat curve follows the general

TABLE 2

The influence of the number of leucocytes in milk on their distribution

Leucocytes added (gm./pint)	Leucocytes per ml. before storage	Leucocytes per ml. after storage in the					
		Upper one-third of pint bottle		Middle one-third of pint bottle		Lower one-third of pint bottle	
		No.	%	No.	%	No.	%
0.0	400,000	1,440,000	71.3	200,000	9.9	380,000	18.8
2.5	9,800,000	16,000,000	94.2	60,000	0.4	920,000	5.4
5.0	20,000,000	44,800,000	93.5	80,000	0.1	3,060,000	6.4
7.5	28,400,000	64,000,000	92.8	160,000	0.2	4,820,000	7.0
10.0	39,600,000	83,200,000	93.5	120,000	0.1	5,660,000	6.4
12.5	48,000,000	111,200,000	95.8	380,000	0.3	4,520,000	3.9
15.0	56,500,000	34,400,000	25.0	2,180,000	1.6	101,000,000	73.4
20.0	74,500,000	32,200,000	16.8	560,000	0.3	159,200,000	82.9
25.0	101,000,000	31,400,000	15.1	340,000	0.2	175,600,000	84.7
30.0	109,500,000	21,700,000	8.7	280,000	0.1	228,800,000	91.2
45.0	175,000,000	11,200,000	2.2	300,000	0.1	492,000,000	97.7
60.0	220,000,000	6,600,000	1.2	60,000	0.0	544,000,000	98.8

direction of the leucocyte curve, although it tends to lag behind the leucocyte curve from the "breaking point" on. However, upon the addition of between 30 and 40 gm. of washed leucocytes, the upper and lower fat curves cross; and thus, beyond the addition of 40 gm. of washed leucocytes, there was more fat in the lower one-third portion of the milk than there was in the upper one-third. From this graph (figure 2) it can be seen also that the fat had a strong tendency to remain in the upper part of the bottle, but that the number of leucocytes evidently finally overcame the buoyancy of the fat.

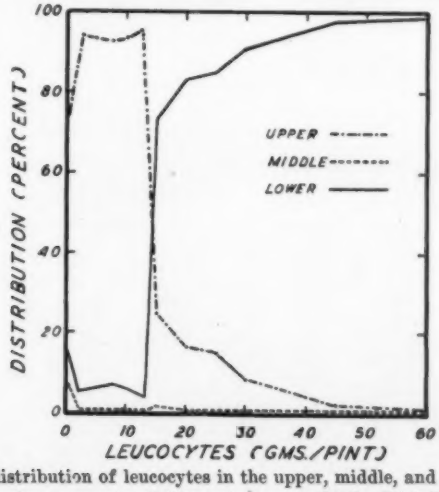

FIG. 1. The distribution of leucocytes in the upper, middle, and lower thirds of pint bottles, to which various amounts of leucocytes were added. Distribution of leucocytes is shown as per cent of total number per bottle.

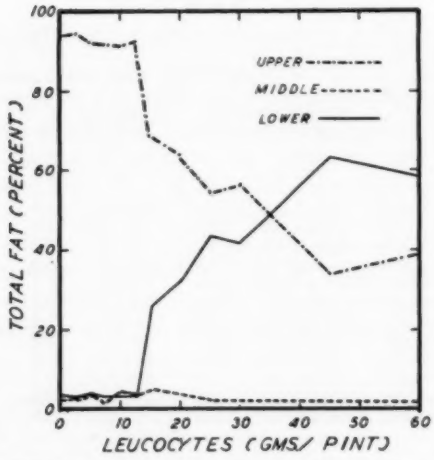

FIG. 2. The distribution of fat in the upper, middle, and lower one-thirds of pint bottles to which various amounts of leucocytes were added.

The hues of the respective cream layers gave further indication of the relative predominance of fat or of leucocytes in the cream. In general the color of the "cream" in the lower layer was of a darker gray hue than that of the upper layer. When Sudan III was added to the milk, both the upper and the lower cream layers showed a greyish-red color upon the addition of increasing weights of leucocytes.

TABLE 3

The influence of the number of leucocytes in milk on the distribution of the fat after storage

Leucocytes added (gm./pint)	Distribution of fat in the								
	Upper one-third of pint bottle			Middle one-third of pint bottle			Lower one-third of pint bottle		
	Fat (%)	Gm. of fat	Per cent of total	Fat (%)	Gm. of fat	Per cent of total	Fat (%)	Gm. of fat	Per cent of total
0.0	10.1	16.463	93.5	0.3	0.489	2.8	0.4	0.652	3.7
2.5	10.1	16.463	94.4	0.3	0.489	2.8	0.3	0.489	2.8
5.0	10.1	16.463	92.6	0.4	0.652	3.7	0.4	0.652	3.7
7.5	10.0	16.300	95.2	0.2	0.326	1.9	0.3	0.489	2.9
10.0	9.7	15.811	91.5	0.5	0.815	4.7	0.4	0.652	3.8
12.5	10.3	16.789	92.8	0.4	0.652	3.6	0.4	0.652	3.6
15.0	7.1	11.573	68.9	0.5	0.815	4.9	2.7	4.401	26.2
20.0	6.7	10.921	63.8	0.4	0.652	3.8	3.4	5.542	32.4
25.0	5.8	9.454	54.2	0.2	0.326	1.9	4.7	7.661	43.9
30.0	5.9	9.617	56.2	0.2	0.326	1.9	4.4	7.172	41.9
45.0	3.5	5.705	34.3	0.2	0.326	2.0	6.5	10.595	63.7
60.0	4.4	7.172	39.6	0.2	0.326	1.8	6.5	10.595	58.6

FIG. 3. The creaming of milk to which various amounts of leucocytes have been added. From left to right respectively additions of 0.0, 7.5, 15, 30 and 60 grams washed leucocytes were made. Sudan III was added for photographic purposes.

DISCUSSION

The results of the above experiment demonstrate that a relatively strong attraction exists between the milk fat globules and the leucocytes found in milk. It would seem that the rising of the leucocytes with the buoyant fat globules cannot be regarded merely as the result of the upward "filtering," "rafting" or "sweeping" action of the fat globules, strong as this buoyant effect may be. This might be the sole contributing force if the leucocytes would be carried up by the rising fat globules and the fat globules would not be pulled down by the excessive numbers of settling leucocytes. The response of the leucocytes to the fat globules and vice versa must therefore indicate the existence, to a very appreciable extent, of a mutual attraction for each other based upon some physico-chemical law. This mutual attraction must serve to explain, in part at least, the abnormally deep cream layers sometimes observed on mastitic milk.

SUMMARY

Adding increasing increments of washed leucocytes to freshly pasteurized, nonhomogenized milk resulted in increasing the depth of the cream layer to its "breaking point" beyond which a "cream layer" composed of fat and leucocytes formed at the bottom of the bottle.

A mutual attraction appears to exist between the milk fat globules and the leucocytes of milk.

REFERENCES

(1) Babcock, C. J. The Effect of Homogenization on Certain Characteristics of Milk. U. S. Dept. Agr. Tech. Bul. 438. 1934.

(2) Trout, G. M., Scheid, M. V., Peters, I. I., and Mallmann, W. L. Influence of Bacteria, Yeast and Leucocyte Distribution in Bottled Homogenized Milk on Sediment Formation. Mich. Agr. Expt. Sta. Quart. Bul. 26(4): 285–296. 1944.

THE INFLUENCE OF pH ON THE ATTRACTION BETWEEN THE FAT GLOBULES AND LEUCOCYTES OF MILK*

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Since previous results by the authors (8) confirmed the belief that a mutual attraction exists between the fat globules and leucocytes of milk, a further study appeared to be of value in order to obtain a better understanding of this behavior. This investigation concerns the influence of pH on the migration of washed leucocytes in milk.

This recognition of the association between the fat and leucocytes of milk is not new. Schuppius (13) observed the presence of up to fifty per cent by volume of fat in the sediment obtained by centrifuging milk by the Trommsdorff method. Later Babcock (1) and Trout and associates (14) reported the migration of leucocytes with the rising fat globules, and attributed this behavior of the leucocytes to the "filtering" or "sweeping" action of the rising fat globules.

Peters and Trout (8) had shown that there existed a very strong attraction between milk fat globules and washed leucocytes. By adding increasing increments of washed leucocytes to pint bottles of nonhomogenized pasteurized milk, they were able to demonstrate the deposition of up to 63.7 per cent of the total fat into the lower one-third portion of the bottle.

Picton and Linder (9) studied the movement of colloidal particles exposed to an electric current. They found that haemoglobin was markedly repelled from the positive electrode. The same authors were able to show in later work (10) that the movement of the particles, when exposed to an electric current, depended upon the chemical nature of the colloidal substances.

Hardy (3) observed the strange behavior of boiled egg white. He noted that under the influence of a constant current the protein particles moved with the negative stream if the reaction of the fluid were alkaline, and with the positive stream if the reaction were acid. He concluded (4) that, since the protein particles had the ability to reverse their electric charge from negative to positive, depending on the alkalinity or acidity of the liquid, there must be a point somewhere along the line at which the particles were isoelectric. This isoelectric point he found to be of great significance.

Prieger (12) determined the isoelectric point of an artificially prepared emulsion made from extracted milk fat and distilled water. She found the isoelectric point of the fat in the artificial emulsion to be more on the acid side than that of the normal fat globules. This difference in the isoelectric point was attributed to the protein layer surrounding the normal fat globules in milk.

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Mommsen (5) found the fat globules of cow's and human milk to carry a negative charge. His findings have been verified by later workers. North and Sommer (7) were able to show the isoelectric point at the fat-serum interface of milk to be at pH 4.3.

According to Bechhold (2) leucocytes carry a negative charge, since they are attracted to the anode. Metschnikoff, as quoted by Bechhold (2), observed a great similarity in the phagocytic action of both leucocytes and amoebae, although the latter carried a positive charge.

Monguio (6) studied the polar movement of leucocytes when exposed to an electric current and concluded that the turning point of the leucocytes varied with the physiological condition of the cell. On the basis of his results he was able to explain the observations made previously by Dineur (Monguio (6)), who found that, at a given strength of current, normal leucocytes gathered at the anode while those from inflamed tissues migrated to the cathode. Thus, since milk from an inflamed or irritated udder normally shows an increased leucocyte count (Plastridge and associates (11)), it leads one to believe that most of the leucocytes found in milk would possess a positive charge. Based upon this assumption the following experiment was undertaken.

Since fat globules carry a negative charge normally (Mommsen (5)), with its isoelectric point at pH 4.3 (North and Sommer (7)), below which point they obtain a positive charge, and the leucocytes were assumed to carry a positive charge, it was felt that, by lowering the pH below the isoelectric point of the fat globules, the behavior of the similarly charged leucocytes toward the fat globules could be observed. If the rising of the leucocytes with the fat were due only to the sweeping action of the fat, then the number of leucocytes migrating to the top of the bottle should be the same in both cases, whether the fat and the leucocytes carried the same or opposite charges. If, however, the rising were due to attraction of opposite charges, then the leucocytes should rise with the negatively charged fat globules, but settle down when the fat carried a positive charge.

EXPERIMENTAL

Pasteurized milk, held for 24 hours at 40° F., was tempered to 80° F. and separated. The cream was diluted with physiological salt solution at 80° F. and separated again. This washing and separation was repeated until the separated physiological salt solution was fairly clear. The skim milk was heated to 86° F., coagulated with rennet, and cooked at 120° F. in order to secure clear whey. After filtering, the whey was heated to 180° F., cooled to 80° F., and then clarified in order to remove all suspended matter.

Washed leucocytes were secured by repeated washing of separator slime in physiological salt solution, centrifuging and decanting.

After standardizing the whey and washed cream to the approximate original fat percentage of the milk, washed leucocytes were added at the rate

of 0.5 gm. per quart. The mixture was then acidified using N/1 HCl, samples being taken at decreasing pH values down to a pH of 1.5. A series of samples ranging in pH from 6.5 to 1.5 were stored in pint bottles for 48 hours at 40° F., after which time examinations were made as to cream volume, sediment formation, distribution of leucocytes and translucency.

RESULTS

The attraction between the fat globules and the leucocytes in the washed-cream, sweet-whey preparation varied markedly depending upon the pH. At pH 4.3, the isoelectric point of the fat-serum interface, fat rising was most complete, and was associated with the greatest numbers of leucocytes, thus leaving a clear, translucent whey below with no sediment at the bottom (figure 2). As the pH values were increased or decreased from pH 4.3, the cream volume was less and was associated with fewer leucocytes (table 1, figure 2) until, at extremes of pH 6.5 or 1.5, the volumes were of comparable size and the whey showed an opaque milkiness with some white sediment at the bottom of the bottles.

TABLE 1

The influence of pH on fat rising in a washed-cream, heat-treated whey mixture

pH of sample	Cream volume	Appearance of underlayer	White sediment layer
	ml.		
6.5	53	Opaque, milky	Slight
6.0	45	Opaque, milky	Slight
5.0	182	Fairly translucent	None
4.3	212	Translucent	None
4.0	170	Fairly translucent	None
3.5	53	Opaque, milky	Very slight
3.0	45	Opaque, milky	Slight
2.5	45	Opaque, milky	Slight
2.0	45	Opaque, milky	Slight
1.5	45	Opaque, milky	Slight

The distribution of leucocytes in the upper, middle, and lower one-third portion of each sample is shown in table 2. In general, fewer leucocytes were associated with the fat as the pH of the mixture departed from pH 4.3. Above the isoelectric point of the fat globules, however, the attraction between the fat and the leucocytes was slightly greater than at the lower pH values. Here the leucocyte count in the cream layer was lowest, indicating that the fat, although rising towards the top, had failed to carry the leucocytes with it. Data in figure 1 illustrate the behavior of the leucocytes with respect to the amount of fat rising. As increasing amounts of acid were added, decreasing the pH, the positive electric charge on the fat globules must have increased, and thus repulsion between the fat globules and the leucocytes became more pronounced, preventing the leucocytes from being carried up by the sweeping action of the rising fat.

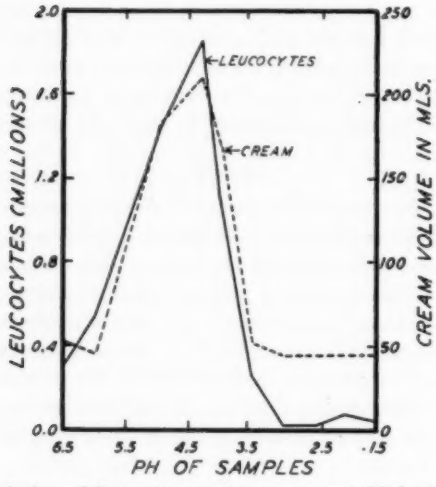

FIG. 1. The relation of leucocyte count in upper one-third of pint bottles to the cream volume in the respective bottles.

The data in figure 1 show that above the isoelectric point of fat the cream-volume curve lags behind the leucocyte curve, indicating that the leucocytes are carried up proportionately more than the fat. At pH values below the isoelectric point, however, the leucocytes are carried up proportionately less than the fat, particularly at pH 3.0 or below, where the difference is most pronounced.

When the pH values of the mixtures were at 6.0, 3.0, 2.5, 2.0, 1.5, the cream volumes were the same, namely, 45 ml.; yet the leucocyte counts in the

TABLE 2

The influence of pH on the migration of leucocytes in a washed-cream, heat-treated whey mixture

pH of sample	Leucocytes per ml. in the					
	Upper one-third of pint bottle		Middle one-third of pint bottle		Lower one-third of pint bottle	
	Number	% of total	Number	% of total	Number	% of total
6.5	300,000	15.6	80,000	4.2	1,540,000	80.2
6.0	540,000	25.5	100,000	4.7	1,480,000	69.8
5.0	1,420,000	83.5	200,000	11.7	80,000	4.8
4.3	1,860,000	88.5	200,000	9.5	40,000	2.0
4.0	1,100,000	80.9	120,000	8.8	140,000	10.3
3.5	260,000	43.3	40,000	6.7	300,000	50.0
3.0	20,000	1.3	40,000	2.6	1,480,000	96.1
2.5	20,000	1.3	20,000	1.3	1,500,000	97.4
2.0	80,000	4.9	40,000	2.4	1,520,000	92.7
1.5	40,000	2.4	10,000	0.6	1,600,000	97.0

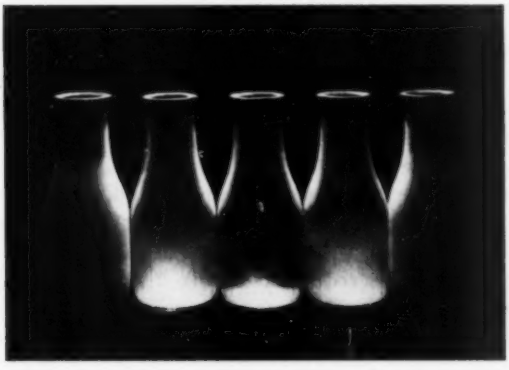

FIG. 2. Pint bottles of washed-cream, heat-treated whey mixtures having pH values from left to right of 6.0, 5.0, 4.3, 4.0, and 3.0. Note the decrease of cream volume in the samples having pH values above and below that at the isoelectric point and also the clearness of whey in the lower portions.

upper one-third portions of the mixture ranged from 540,000 to 20,000 per ml. (tables 1 and 2). Assuming that comparable fat rising with clumping had taken place at these pH values, then it would follow that equal numbers of leucocytes might be expected in the two cream layers. The reason for the difference in the behavior of the leucocytes or the fat must then be due to the fact that both carry the same electric charge and thus tend to repel each other. This would tend to explain the decreasing number of leucocytes carried into the upper portions with decreasing pH values.

FIG. 3. Pint bottles of washed-cream, heat-treated whey mixtures ranging in pH from 6.5 to 2.5 from left to right. (1) pH 6.5—normal range. Fat carries a negative charge. (2) pH 4.3—isoelectric point of fat. No charge. (3) pH 2.5—high-acid range. Fat carries a positive charge. Note the cream volumes and the similarity of opacity of the lower portions in bottles 1 and 3.

DISCUSSION

The rising of the washed leucocytes with the negatively charged fat globules and their failure to rise with the positively charged fat globules indicate that the attraction between the washed leucocytes and the fat globules must be based, in part at least, on electric attraction rather than on the sweeping or filtering action of the rising fat globules.

The assumption that the leucocytes found in milk carry a positive charge seems to be true, according to the results obtained in the above experiment. The presence of bacteria in the udder would attract leucocytes of a phagocytic nature which, as observed by Metschnikoff, behave similar to amoebae, the latter carrying a positive charge. Since milk from irritated or inflamed udders contains relatively large numbers of leucocytes, they too must carry a positive charge.

The results of the few trials conducted may not be sufficient evidence to make any definite conclusions. Nevertheless, they serve as a good indication that the attraction between the fat globules and the washed leucocytes is based upon opposite electric charges which are attracted to each other, and thus allow for the rising or settling of the leucocytes with respect to the fat.

SUMMARY

Lowering the pH of a washed-cream, heat-treated-whey mixture containing added washed leucocytes from pH 6.5 to 1.5 resulted in marked differences in the attraction between the fat and the leucocytes. Migration of the washed leucocytes with the rising fat globules was greatest at pH 4.3, the isoelectric point of the fat globules. Below this value the attraction between the fat globules and the leucocytes was not as great as when the pH was above that of the isoelectric point. Thus, when the electric charge of the fat globules was reversed from negative to positive, the washed leucocytes did not respond so readily to the "sweeping" action of the fat.

The results indicate that the attraction of the fat globules and the washed leucocytes is based, in part at least, upon the opposite electric charges carried by the fat globules and the leucocytes found in milk.

REFERENCES

(1) BABCOCK, C. J. The Effect of Homogenization on Certain Characteristics of Milk. U. S. Dept. Agr. Tech. Bul. 438. 1934.

(2) BECHHOLD, H. Die Kolloide in Biologie und Chemie. Ed. 5, 568 pp., illus. Dresden und Leipzig: von Theodor Steinhogf. 1929.

(3) HARDY, W. B. On the Coagulation of Proteid by Electricity. Jour. Physiol., 24: 288. 1899.

(4) HARDY, W. B. Eine vorlaüfige Untersuchung der Bedingungen 'welche die Stabilität von nicht umkehrbaren Hydrosolen bestimmen. Ztschr. f. Phys. Chem. 33: 385. 1900.

(5) MOMMSEN, H. Über die Kataphorese von Kuhmilch und Frauenmilch. Monatsschr. f. Kinderheilk, 42: 361. 1932.

(6) MONGUIO, J. Über die polare Wirkung des galvanischen Stromes auf Leukozyten. Ztschr. f. Biol., **93**: 553. 1933.

(7) NORTH, G. C., AND SOMMER, H. H. Electrokinetics in Relation to Dairy Phenomena. JOUR. DAIRY SCI., **18**: 21–43. 1935.

(8) PETERS, I. I., AND TROUT, G. M. The Attraction between the Fat Globules and the Leucocytes of Milk. JOUR. DAIRY SCI., **28**: 277–289. 1945.

(9) PICTON, H., AND LINDER, S. E. Solution and Pseudo-Solution. Jour. Chem. Soc., **61**: 148. 1892.

(10) PICTON, H., AND LINDER, S. E. Solution and Pseudo-Solution. Part III. The Electrical Convection of Certain Dissolved Substances. Jour. Chem. Soc., **71**: 568. 1897.

(11) PLASTRIDGE, W. N., ANDERSON, G. O., WILLIAMS, L. F., AND WEIRETHER, F. J. Infectious Bovine Mastitis. Storrs Agr. Expt. Sta. Bul. 231. 1939.

(12) PRIAGER, I. Über die Hüllen der Milchfettkügelchen. Ztschr. Biochem., **217**: 331. 1930. (Abs., Koll. Ztschr., **54**: 351, 1931. Original not seen.)

(13) SCHUPPIUS, R. Die Milchleukozytenprobe nach Trommsdorff. Arch. f. Hyg., **62**: 137–146. 1907.

(14) TROUT, G. M., SCHEID, M. V., PETERS, I. I., AND MALLMANN, E. L. Influence of Bacteria, Yeast and Leucocyte Distribution in Bottled Homogenized Milk on Sediment Formation. Mich. Agr. Expt. Sta. Quart. Bul. **26** (4): 285–296. 1944.

COBALT DEFICIENCY IN CATTLE IN THE NORTH EASTERN REGION OF WISCONSIN*

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Cobalt deficiency in cattle and sheep has been reported for a number of regions in the world including Australia (4, 5, 9), New Zealand (1), Scotland (3), Florida (7), and Michigan (2). Recently, our attention was brought to this problem in Door County, Wisconsin, by Mr. G. I. Mullendore, County Agent of Door County, Wisconsin, and Mr. Evan Hirsch, foreman of the Door County Branch Station farm. The area of deficiency involves a greater area than Door County, but the boundaries have not yet been determined. Unthriftiness and emaciation were reported in bulls, cows, and calves in certain herds in that region. Because of the occurrence of a cobalt deficiency in cattle in the Grand Traverse Region of Michigan (2), characterized by symptoms not different from those observed in cattle in the Door County area, we turned to a study of the blood of animals in these afflicted herds.

EXPERIMENTAL

The blood samples were drawn from the animals directly on the various afflicted farms in Door County and surrounding counties, were citrated immediately, and then sent to the laboratory in time for analysis on the following day. Samples showing evidence of clotting were discarded, and the hemoglobin values were determined with the aid of the Evelyn photoelectric colorimeter. Blood analyses were again made on the same animals at the end of two and five months.

At the time of sampling, the physical condition of the animals was noted; and, where possible, the character and source of the feed constituents were ascertained. Cobalt therapy was started simultaneously and was that recommended by the Michigan workers (2), that is, one ounce of cobalt sulfate ($CoSO_4 \cdot 7H_2O$) per 100 pounds of salt or one teaspoonful daily of a solution made by dissolving one ounce of either cobalt sulfate or cobalt chloride ($CoCl_2 \cdot 6H_2O$) in one gallon of water. With the use of a salt-cobalt mixture constituting 1 per cent of the grain ration, and the daily grain consumption calculated at 5 to 10 pounds, the amount of elemental cobalt ingested varied from 3 to 6 mg. per day, or in other words, about 0.6 mg. of cobalt per pound of grain consumed. One teaspoonful of the solution supplied 7 to 9 mg. of elemental cobalt daily.

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The authors wish to thank Dr. Norman Lundquist for obtaining many of the blood samples from the University herd.

TABLE 1

Effect of cobalt therapy on the hemoglobin level and physical condition of cobalt deficient cattle

Farm number	Animal	Cobalt therapy, mg./day	Hemoglobin value, grams per 100 cc.					Relative physical improvement
			9/23/43	10/19/43	12/22/43	3/18/44	5/9/44	
1	Calf	None*	.....	7.30	8.91	9.47	9.80*	Good
	Calf	7–9‡	.....	7.40	9.47	10.97	11.37	Good
2	Calf, small	7–9‡	.....	7.60	9.41	9.41	9.73†	Very good
	Calf, large	7–9‡	.....	7.12	10.25	11.05	8.99†	Very good
	Calf, large	None	.....	10.97	10.35	10.04	9.73	Little change
3	Guernsey Bull (4 yrs.)	7–9‡	.....	6.24	9.47	.....	.....	Excellent
4	Calf	3–6§	7.75	6.11	9.65	.....	11.10	Very good
	Calf	3–6§	7.70	7.90	10.45	.....	10.09	Very good
5	Bull calf (10 months)	3–6§	5.75	8.50	9.26	.....	.....	Good
6	Holstein calf	7–9§	.....	.....	4.97	.....	10.66	Very good
	Bull (2 yrs.)	7–9§	.....	.....	7.59	.....	11.71	Very good

* Fed cobalt beginning March 20, 1944.
† Cobalt discontinued March 20, 1944.
‡ Cobalt solution added to drinking water.
§ Cobalt sulfate added to salt.

Data on the animals and the effects of cobalt therapy are presented in table 1. Figures 1 and 2 show the physical appearance of a Holstein calf from Farm 6 before and after the feeding of cobalt.

In extending this problem, it was decided to determine what would be considered a normal hemoglobin level of cattle blood. This work was done with animals of the University herd, and yielded the following results.

1. Eight calves (Holsteins), about 7 months old, showed hemoglobin values ranging from 9.6 to 10.4 grams per 100 cc.

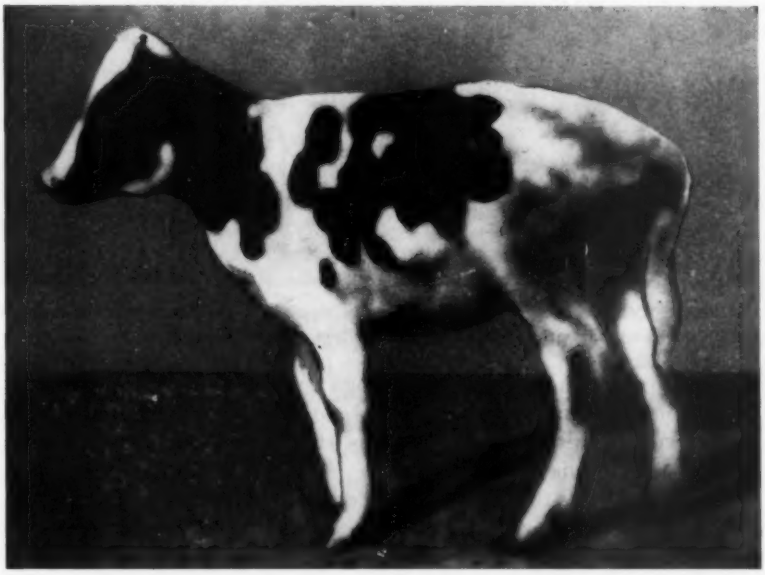

FIG. 1. Holstein calf at Farm No. 6, December 22, 1943. Age 7 or 8 months; eating very poorly, preferred straw to hay. Hemoglobin reading 4.97. Started cobalt in drinking water.

2. Fourteen heifers (Holsteins), about one year old, showed hemoglobin values ranging from 9.7 to 12.6 grams per 100 cc. Only one individual showed as high a hemoglobin level as 12.5. Excluding that animal, the range was from 9.7 to 11.8. The greater number fell between 9.7 and 10.9.

3. Eleven mature milking cows showed a hemoglobin range of from 10.04 to 11.8 grams per 100 cc. of blood.

From these data it is evident that age is not a significant factor concerned with the hemoglobin level of cattle 7 or more months old, and that the normal range for such cattle is from 9.5 to 12 grams of hemoglobin per 100 cc. of blood.

Since it is always possible that, in practice, excessive amounts of a therapeutic agent may be used, studies were made on the tolerance levels of cobalt

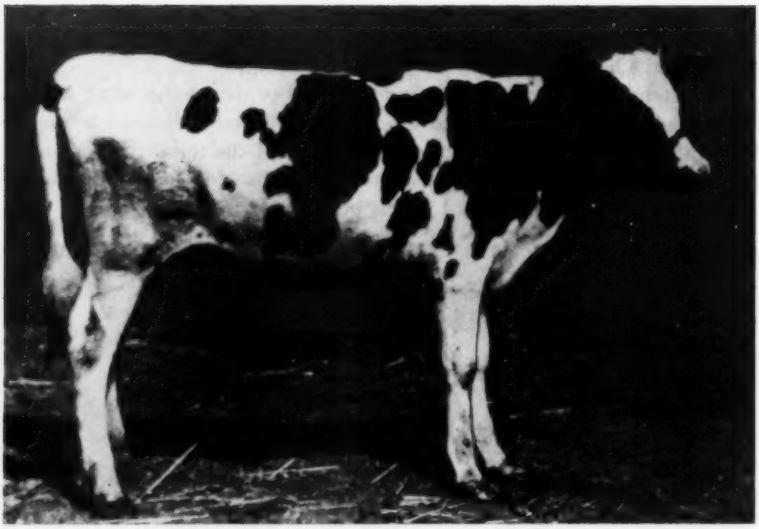

FIG. 2. May 9, 1944. Same animal as in figure 1. Condition greatly improved. Calf appeared normal with excellent appetite. Hemoglobin reading 11.71.

by cattle to determine whether or not high levels of this element would produce an unusual rise in the hemoglobin and number of erythrocytes. It is well known that, with the rat, as little as 0.05 to 0.1 mg. of cobalt daily will cause a polycythemia (6, 8, 10).

For these studies three Holstein heifers weighing about 500 pounds each were chosen. Hematocrits and R.B.C. counts were run only occasionally because work with the rat has shown that the hemoglobin level is a good index of the polycythemia induced by excessive cobalt (8). Hemoglobin

TABLE 2

Effect of cobalt feeding on the hemoglobin and hematocrit values of cattle blood. A, B, and C each represent one Holstein heifer

Week	Elemental cobalt, mg./day/ animal	Hemoglobin,* grams/100 cc.			Hematocrit, per cent			R.B.C. count per mm.³		
		A	B	C	A	B	C	A	B	C
1	0	10.11	8.71	9.36	47	41	43	6,350,000	6,320,000	7,340,000
2	3	10.70	8.63	9.53	.....	.....	.....	.....	.....	.....
3	6	10.04	7.93	9.09	45	36	41	.....	.....	.....
4	9	9.86	8.17	8.73	.....	.....	.....	.....	.....	.....
5	20	10.04	8.36	8.81	.....	.....	.....	.....	.....	.....
6	20	9.80	8.54	9.15	.....	.....	.....	.....	.....	.....
7	50	10.65	8.95	9.51	.....	.....	.....	.....	.....	.....
8	50	10.85	9.49	9.88	50	45	42	.....	.....	.....
9	50	10.73	9.02	9.56	.....	.....	.....	.....	.....	.....

* Each figure represents average of values on 3rd and 7th days of each week.

determinations were made on the third and seventh days of each week throughout the experiment. To insure the consumption of a definitely known amount of cobalt each day, gelatin capsules containing the correct amount of cobalt sulfate ($CoSO_4 \cdot 7H_2O$) were employed. Each animal received one capsule early each day. The results are given in table 2.

DISCUSSION

Table 1 and figures 1 and 2 clearly show that a cobalt deficiency exists in Northeastern Wisconsin, and that cobalt therapy is very effective in correcting this condition. On Farm 3 a Guernsey bull showed not only a great improvement in physical appearance and hemoglobin level, but also a gain of 150 pounds in 2 months. Cobalt therapy was effective, also in the case of a young spring calf from Farm 6, whose hemoglobin value was 4.97 (see figures 1 and 2). When given cobalt, this animal made rapid physical improvement, and at the same time the hemoglobin level increased. As stated previously, the deficiency was observed in cattle of all ages. Not every individual in a herd was afflicted, and in addition, there was a seasonal variation. More deficiencies were observed in late fall, winter, and early spring than when the animals were on pasture.

It was observed that hemoglobin building was slow when cobalt was given, while appetite and general well-being were rapidly restored. Whether there are other deficiencies such as iron and copper involved in the nutrition of these herds has not been ascertained. The cobalt treatment did restore the animals, and the hemoglobin level came back to normal after a month or so of therapy; but it is possible that a mixture of iron, copper, and cobalt would have brought the hemoglobin level to normal in less time. Future experiments must answer this question.

Not all of the animals studied were from Door County; some hemoglobin determinations were made on animals in adjoining counties and showed the same deficiency picture. The establishment of any set boundaries for the cobalt-low area must wait until many determinations are made throughout the state. Because the animals on the various farms received rations which differed in quality, composition, and source of ingredients, clear cut results were not always obtained, nor should the same response to cobalt feeding be expected for all animals. Table 1 shows that the control animal on Farm 1 respondedwithout treatment although it never did reach the level of the treated animal. This "spontaneous" cure may have been due in part to the introduction of concentrates from outside the deficient area during the period of observation.

The results of the work done to ascertain the normal hemoglobin level in cattle show the relative unimportnace of age in affecting this level, but it should be noted that no animals below 7 months of age were tested. What little upward trend was noticed as the age increased was offset by the overlapping between the various age groups.

It can be safely concluded that the amounts of cobalt given in therapy are far below any level required to induce polycythemia, if any such level does exist. Table 2 demonstrates that as much as 50 mg. may be given daily without raising the hematocrit or hemoglobin values. The total amount of cobalt fed during the 8 weeks of cobalt feeding was 1.456 grams. This means that for the 56-day period each animal received 26 mg. daily. Calculated on the basis of the therapeutic level of 3 mg. per day, this amount would have been sufficient for a period of 485 days of therapy.

SUMMARY

1. Cobalt deficiency, characterized by unthriftiness and anemia, has been observed in cattle in Northeastern Wisconsin.

2. This deficiency was corrected by the feeding of salt containing one ounce of cobalt sulfate ($CoSO_4 \cdot 7H_2O$) per 100 pounds. When this salt mixture is incorporated into the grain at a level of one per cent, it will supply about 3 mg. of elemental cobalt per day when five pounds of grain are consumed. Good results were also secured by the daily feeding of one teaspoonful of a solution made by dissolving one ounce of either cobalt sulphate or cobalt chloride in one gallon of water.

3. The normal range of the hemoglobin level of calves, heifers, and cows was 9.5 to 12.5 grams per 100 cc. of blood.

4. The tolerance for cobalt in this species was high. Feeding as much as 50 mg. of cobalt per day produced no polycythemia.

REFERENCES

(1) Askew, H. O., and Dixon, J. K. The Importance of Cobalt in the Treatment of Certain Stock Ailments in South Island, New Zealand. New Zeal. Jour. Sci. and Technol., **18**: 73. 1936.

(2) Baltzer, O. C., Killhan, B. J., Duncan, C. W., and Huffman, C. F. A Cobalt Deficiency Disease Observed in Some Michigan Dairy Cattle. Mich. Agr. Expt. Sta. Quart. Bul., **24**: 68. 1941.

(3) Dunlop, G., and McCalien, W. J. Influence of Geology on the Health of Grazing Animals. Nature, **147**: 615. 1941.

(4) Lines, E. W. The Effect of the Ingestion of Minute Quantities of Cobalt by Sheep Affected with "Coast Disease": A Preliminary Note. Jour. Council Sci. and Indus. Res. (Austral.), **8**: 117. 1935.

(5) Marston, H. R. Problems Associated with "Coast Disease" in South Australia. Jour. Council Sci. and Indus. Res. (Austral.), **8**: 111–116. 1935.

(6) Mascherpa, P. Le Pouvoir Hematopoietique du Cobalt. Arch. Ital. de Biol., **82**: 112. 1930.

(7) Neal, W. M., and Ahmann, C. F. The Essentiality of Cobalt in Bovine Nutrition. Jour. Dairy Sci., **20**: 741. 1937.

(8) Stare, F. J., and Elvehjem, C. A. Cobalt in Animal Nutrition. Jour. Biol. Chem., **99**: 473. 1933.

(9) Underwood, E. J., and Filmer, J. F. The Determination of the Biologically Potent Element (Cobalt) in Limonite. Austral. Vet. Jour., **11**: 89. 1935.

(10) Waltner, K., and Waltner, K. Kobalt und Blut. Klin. Wchnschr., **8**: 313. 1929.

THE EFFECT OF THYROIDECTOMIZED GOAT'S MILK ON THE HEAT PRODUCTION OF NORMAL AND HYPERTHYROID RATS[1]

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Since 1906 fragmentary evidence has been accumulating, chiefly from clinical reports, that the milk of thyroidectomized animals contains an anti-thyroid principle. Little is known of the nature or mode of action of this substance but its presence has been postulated in an attempt to explain various physiological and clinical observations.

Evidence indicates that two principles, one anti-thyrotropic and one anti-thyroxin, may be involved in this activity. It has been postulated that the antagonistic principle is a normal constituent of the blood and is so balanced under normal conditions that its presence is not evident. Only after an imbalance occurs, due to a reduction of the normal concentration of the hormone or an increase in the relative amount of its antagonist, is the inhibitory substance revealed.

The alleviation of the hyperthyroid symptoms of goitrous patients might be elicited by one of two possible hormonal activities, either by an indirect inhibition of thyroxin production by an anti-thyrotropic principle or directly by some anti-thyroxin principle.

A method for demonstrating a specific anti-hormone in blood serum has been proposed by Collip (3). This method consists of pretreatment of the subject with the serum or serum extract to be tested, followed by the simultaneous injection of a known amount of hormone and serum or serum extract. Consistent inhibition of the hormone effect by the anti-hormone serum, as compared with suitable controls, indicates the presence of an anti-hormone substance. (In the study reported herein a similar procedure was followed except that milk was tested instead of blood serum.)

If the foregoing postulation is valid it would seem logical to expect an imbalance to occur between thyroxin and its antagonist upon thyroidectomy of the animals. It is also logical to assume, on the same basis, that an imbalance could conceivably be produced between the thyrotropic hormone of the anterior pituitary and its inhibitory principle by a similar operation. If such were the case, it would likewise be conceivable that the inhibitory principles in the blood could be carried over into the milk of the lactating, thyroidectomized animal. The milk of these animals could be then fed and

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the inhibitory substance present would limit the amount of available thyroxin in the tissues, thus causing a decrease in the metabolic rate.

EXPERIMENTAL

The respiration apparatus used to determine the metabolic rates in this study was a closed-system type patterned, with some minor modifications, after the one described by Marquand (9) in which oxygen consumption is measured directly and carbon dioxide production indirectly by absorption in standard sodium hydroxide and the excess titrated against standard hydrochloric acid. It had a large oxygen reservoir patterned after Wesson (14) and an absorbing system as suggested by Garrison (5). The rats were placed in an incubator at 30° C. for 24 hours before the metabolism tests were made and were held at this temperature during the tests by means of a constant temperature water bath. At this temperature the heat production of rats is not affected by the environmental temperature according to Benedict and McLeod (1).

The R.Q. was determined directly in all cases except in Experiment 3 where an average R.Q. of 0.84 was assumed. This assumption was made on the basis of the average R.Q. in Experiment 2. It was found that the variation was so small that to assume an average of 0.84 would cause an error of less than one per cent in the final B.M.R.

In order to check our ability to determine changes in metabolic rate a few rats were hypophysectomized after basal metabolism determinations had been made. As would be expected, heat production decreased about 35 per cent. After injecting small doses of thyroxin heat production rose to approximately normal.

Experiment 1. In a preliminary study of the effect on heat production of feeding thyroidectomized goats' milk to normal rats, a group of 16 mature female rats was selected. Post-absorptive basal metabolism determinations were made weekly for four weeks and the average was considered to be the rats' normal basal metabolic rate. These rats were then divided at random into two groups of eight. Group I was placed on the regular stock ration *ad libitum* plus normal goats' milk *ad libitum* and Group II on the same stock ration plus thyroidectomized goats' milk. Metabolism determinations were continued at weekly intervals for six weeks. At the end of this period the group fed thyroidectomized goats' milk showed a slightly lower average basal metabolism. The results of this study are shown in table 1.

Experiment 2. Since the clinical evidence of the beneficial effects of feeding thyroidectomized goats' milk was obtained when such milk was fed to hyperthyroid patients it was decided to produce a similar condition in rats and study the effects of feeding thyroidectomized goats' milk while the rats were in a hyperthyroid state.

Three groups of eleven mature litter-mate rats each were selected. In

TABLE 1

The effect of feeding normal and thyroidectomized goat's milk on the heat production of normal female rats

Group I—Normal goat's milk				Group II—Thyroidectomized goat's milk			
Normal B.M.R.*	B.M.R. after milk feeding	Difference	Difference, per cent	Normal B.M.R.	B.M.R. after milk feeding	Difference	Difference, per cent
621	639	+18	+2.9	637	626	−11	− 1.7
646	624	−22	−3.4	665	605	−60	− 9.0
596	648	+52	+8.7	675	668	− 7	− 1.0
678	664	−14	−2.1	687	687	0	0.0
584	638	+54	+9.2	605	688	+83†	+12.0
666	722	+56	+8.4	656	634	−22	− 3.3
681	694	+13	+1.9	679	656	−23	− 3.4
727	667	−60	−8.2	680	697	+17	+ 2.5
650	662	+12.1	+2.2	661	658	− 2.9	− 0.5

* B.M.R.—Basal metabolic rate expressed in calories per square meter of body surface per 24 hours.

† If this rat is excluded from the averages the average difference becomes −15.1 per cent.

order to insure maximum consumption of milk, the groups of rats were fed exclusively and *ad libitum* on cows' milk, normal goats' milk, and thyroidectomized goats' milk, respectively. The normal and thyroidectomized goats' milk were obtained from the same goats used in the previous study. After establishing the normal basal metabolism by determinations run on

TABLE 2

The effect of feeding normal cow's milk, normal goat's milk and thyroidectomized goat's milk on the heat production of rats with experimental hyperthyroidism

Group I—Cow's milk				Group II—Normal goat's milk				Group III—Thyroidectomized goat's milk			
Normal B.M.R.	B.M.R. after inj.	Increase	Increase, per cent	Normal B.M.R.	B.M.R. after inj.	Increase	Increase, per cent	Normal B.M.R.	B.M.R. after inj.	Increase	Increase, per cent
685	1422	737	108	704	1194	490	70	771*	971*	164*	21*
671†	805†	134†	20†	681	1495	808	117	707	1208	501	71
685	1234	549	80	754	1217	463	60	682	1135	395	58
.....	Died	Died	Died	660	1293	635	96	680	1275	595	87
695	1106	411	59	698	1320	622	89	725	1259	533	73
697	1337	640	92	700	1402	702	100	701	1211	510	73
696	1457	761	109	708	1294	587	83	715	1184	470	66
708	1074	366	52	717	1176	459	64	721	1184	464	64
714	1328	614	86	675	1217	542	80	702	1179	477	68
713	1070	357	50	766	1296	530	70	728	1112	384	53
709	1296	586	82	721	1497	776	107	725	1236	512	71
706	1258	573	80	708	1309	601	85	709	1198	484	68

* Omitted from averages—Died one hour after last determination.

† Omitted from averages—Poor physical condition—scours.

the first and eighth days of the milk feeding period, experimental hyperthyroidism was induced in all three groups by subcutaneous injections of crystalline thyroxine in alkaline solution. Beginning on the ninth day of the feeding period 0.2 mg. of thyroxin per 100 gm. of body weight was injected daily for 6 days, at the end of which time both milk feeding and injections were terminated. On the day following the last injection the post absorptive metabolic rate was again determined.

As shown in table 2 the metabolic rates of all three groups showed a marked increase but all did not rise to the same level. The metabolic rates of the rats in Group I (cows' milk) showed an average increase of 80 per cent over the normal value. The rats in Group II (normal goats' milk) showed an average increase of 85 per cent. However an analysis of variance showed no significant difference between these groups. The average increase in Group III (thyroidectomized goats' milk) was only 68 per cent over the normal value. An analysis of variance between Group III and Group II showed a significant difference as did a similar comparison between Group III and Groups I and II combined.

It is of interest to note that in Group III the greatest increase in heat production was 87 per cent above normal, which was the only value out of 10 above 73 per cent. In the other two groups the greatest increase in heat production was 117 per cent and 7 out of 20 were more than 90 per cent above normal.

Experiment 3. Considering the massive dosage of thyroxin injected in Experiment 2 it seemed reasonable to expect that had the dosage been adjusted to a lower level, a more complete inhibition of its effect would have resulted. Also consideration needed to be given to the fact that the goats used to supply the thyroidectomized goats' milk had been thyroidectomized more than a year before the experiment was begun and that if the effect were transitory, a greater inhibition might have been induced by milk obtained earlier in the post-operative period.

With these points in mind another experiment was set up in which three pairs of male and 1 pair of female rats were used. A recently thyroidectomized goat was used to supply the thyroidectomized goat's milk and this milk was compared with a normal Holstein cow's milk.

After a normal basal metabolic rate had been established one group was fed cow's milk *ad libitum* and the other group received thyroidectomized goat's milk *ad libitum*. On the fourth day of feeding, 0.1 mg. of crystalline thyroxin in alkaline solution was injected subcutaneously daily into the rats of both groups for 10 days, at the end of which time the injections were terminated, but the milk feeding was continued. On the day following the last injection the post-absorptive metabolic rate was determined. Seven days later the metabolic rate was again determined and the experiment was concluded.

The metabolic rate of the first rat in each group was measured without the usual 24-hour starvation period. As would be expected, the heat production of these rats was much higher than that of their respective groups but the rats in Group II (thyroidectomized goat's milk) increased in metabolic rate only 80.1 per cent while the rats in Group I (cow's milk) increased 130.7 per cent. The average increase in Group I (cow's milk) was 94.3 per cent. In Group II (thyroidectomized goat's milk) the average increase was 78.7 per cent. Thus the rats in Group II had a 15.6 per cent lower metabolic rate than those in Group I. This difference was shown to be statistically significant.

One week later, during which no injections were made but the milk feeding was continued, the rats in Group II were found to have an average metabolic rate 1.6 per cent below the normal level while those in Group I were still 14.8 per cent above the normal level, a difference of 16.4 per cent. This difference might have been even greater if one of the rats in Group II had not developed an infected penis.

The results of this experiment are given in table 3.

TABLE 3

The effect of feeding normal cow's milk and thyroidectomized goat's milk on the heat production of rats with experimental hyperthyroidism

Group I—Normal cow's milk					Group II—Thyroidectomized goat's milk				
Normal B.M.R.	B.M.R. after inj.	Increase, per cent	B.M.R. after 1 week	Increase, per cent	Normal B.M.R.	B.M.R. after inj.	Increase, per cent	B.M.R. after 1 week	Increase, per cent
♂ 797	1839	130.7*	982	+23.0	♂ 754	1358	80.1*	948	+26.0
♂ 794	1501	89.0	864	+ 8.8	♂ 791	1298	64.1	599	−24.3
♂ 803	1489	85.4	848	+ 5.6	♂ 780	1258	61.3	761	− 2.5
♀ 691	1189	72.1	841	+21.7	♀ 680	1424	109.4	650	− 4.4
749	1505	94.3	884	+14.8	751	1335	78.7	739	− 1.6

* Not starved.

DISCUSSION

As early as 1906 Möbius (10), working with thyroidectomized sheep, found that the blood serum of such animals neutralized the action of the thyroid hormone. From this work emanated the idea that inhibitory substances can be produced in the body which circulate in the blood stream and neutralize the effects of certain hormones. This work was later confirmed by Blum (2) who in addition found that thyroid hormone inhibitory substances exist in the blood of normal sheep.

According to Collip (3) Saegesser was able to neutralize thyroxin in the Reid-Hunt acetonitril test, by the addition of the blood serum of myxedematous patients. This substance is lipoid-soluble. This is additional evidence of an anti-thyroxin substance. Others have presented evidence

that there is, in addition to the anti-thyroxin substance, an anti-thyrotropic principle. Collip (3) and Collip and Anderson (4) have shown convincing evidence of the existence of an anti-thyrotropic principle which can be produced in the blood stream by the injection of thyrotropic hormone. The refractoriness which develops in animals thus treated is believed to be due to the development of an anti-thyrotropic substance.

There is some disagreement among workers as to whether these inhibitory substances are truly anti-hormones or whether they are immune responses to the proteins associated with the hormones. Werner (13) has presented evidence that the development of refractoriness in rats injected with thyrotropic hormone may depend upon the type of extract in which the hormone is administered. Kin (7) has reported some work which sheds some light on the origin of the "contra thyroid hormone." He has found an ether-soluble substance in the pancreas, which is not present in other glandular organs and which inhibits the lipase reducing power of thyroxin. It also antagonized the Reid-Hunt reaction. This substance decreases markedly in the blood after pancreatectomy and increases after the transplantation of pancreatic tissue.

Depending upon the experimental procedure employed, evidence of the presence of both anti-thyroxin and anti-thyrotropic substances, which are normally present or develop under certain condition such as thyroidectomy, has been presented.

Evidence that these inhibitory substances appear in the milk of thyroidectomized goats was shown in 1935 by Lopez (8) who reported on several cases of exophthalmic goiter in which the hyperthyroid symptoms were alleviated by feeding milk from thyroidectomized goats. The cases reported had refused surgical treatment and all medical methods had been tried in vain or with insignificant results. When milk from thyroidectomized goats was fed to these patients for varying lengths of time remarkable recoveries ensued in nearly all cases.

In the three experiments here reported we have attempted to determine the effect of feeding thyroidectomized goats' milk on the basal metabolism of rats. The results in table 1 show a slightly lower metabolic rate in the group fed thyroidectomized goats' milk. While this is a rather small difference, it is possible that had these rats received an exclusive milk diet the difference might have been greater. However, if the data on one of the rats in Group II whose performance during the entire milk feeding period did not conform to the general pattern of the other animals of this group are omitted, the average difference in metabolic rate for Group II becomes −15.1 and is significantly lower than the average difference for Group I. On this basis it can be said that thyroidectomized goats' milk depressed the basal metabolic rate of normal rats. Some of the wide variation of individual rats is likely due to estrus or uncontrollable activity.

When experimental hyperthyroidism was induced concurrently with the feeding of milk from the same goats used in Experiment 1, a marked difference occurred in the heat production of Group III, as compared to Groups I and II, as shown in table 2. This is interpreted as evidence that the anti-thyroid substance is present in the milk of thyroidectomized goats. The fact that heat production did not rise to as high a level when thyroidectomized goats' milk was fed as it did when normal goats' or cows' milk was fed is considered evidence that the inhibitory substance is anti-thyroxin in nature rather than anti-thyrotropic. This view is also supported by the data in Experiment 1. This evidence does not, however, entirely exclude the possibility of some anti-thyrotropic activity but any such activity is over-shadowed by the anti-thyroxin effect even though it may have been in force.

The results obtained in Experiment 3 were of a confirmatory nature but in addition exhibited more convincing evidence of the presence of an anti-thyroxin substance in the thyroidectomized goats' milk. The fact that the rats in Group II returned to normal before those in the other group further indicates the supressing action of this substance.

Although little has been done in applying this principle to the treatment of toxic goiter in humans, Velde (11) reports that one hundred cases of hyperthyroidism were treated with serum from thyroidectomized animals. In 70 per cent the metabolic rate diminished; 82 per cent showed an increase in weight and 90 per cent obtained clinical amelioration. The amelioration, if treatment was continued several months or one to two years and then stopped, was lasting. Neither exophthalmos nor the size of the goiter decreased.

On the basis of these experiments it would seem desirable to explore further the value of thyroidectomized goats' milk in alleviating the symptoms of toxic goiter in man.

CONCLUSIONS

Thyroidectomized goats' milk was found to have an inhibitory action on heat production in both normal rats and rats with experimental hyperthyroidism. This effect is attributed to the presence of an anti-thyroxin principle in the milk of thyroidectomized goats.

REFERENCES

(1) BENEDICT, F. G., AND MCLEOD, G. The Heat Production of the Albino Rat. II. Influence of Environmental Temperature, Age and Sex; Comparison with Basal Metabolism of Man. Jour. Nutrit., 1: 367. 1928.

(2) BLUM, F. Über die antithyroidalen Eigenschaften des Blutes und das zugrundeliegende Katechin. Schweiz. Med. Wochnschr., LXIII: 777. 1933. Cited by Collip, J. B. Recent Studies on Anti Hormones. Ann. Int. Med., 9: 150. 1935.

(3) COLLIP, J. B. Recent Studies on Antihormones. Ann. Int. Med., 9: 150. 1935.

(4) COLLIP, J. B., AND ANDERSON, E. M. The production of Serum Inhibitory to the Thyrotropic Hormone. The Lancet, 1: 76. 1934.

(5) GARRISON, A. D. Jour. Clin. Med., 17: 814. 1932. Cited by Marquand, C. B. An Experimental Study of Glucose Formation from Fat in the White Rat Using a Modified Respiration Apparatus. Abst. of Doctors Dissertations No. 22. The Ohio State University Press. 1937.

(6) KIN, R. The Antithyroid Substance of Serum. Jour. Chosen Med. Assoc., 28: 151. 1938. (Chem. Abstr., 33: 1806. 1939.)

(7) KIN, R. Antithyroid Substance in Organs. Jour. Chosen Med. Assoc., 28: 1643. 1938. (Chem. Abstr., 33: 1342. 1939.)

(8) LOPEZ, C. P. Six Cases of Exophthalmic Goiter Treated with Milk of Thyroidectomized Goats. La Presna Med. Argent., 22: 1340. 1935.

(9) MARQUAND, C. B. An Experimental Study of Glucose Formation from Fat in the White Rat Using a Modified Respiratory Apparatus. Abstracts of Doctors Dissertations No. 22. The Ohio State University Press. 1937.

(10) MÖBIUS, P. J. Die Basedowache Krankheit. A. Holder Wein. Cited by Collip, J. B. Recent Studies on Antihormones. Ann. Int. Med., 9: 150. 1935.

(11) VELDE, JEAN VANDE. Study of the Thyroid. I. Effect of Treatment by Antithyroid Serums. Rev. Belge. Sci. Med., 11: 20. 1939. Chem. Abstr., 33: 5070. 1939.

(12) WERNER, S. C. Prolonged Injection of Thyrotropic Extract without Development of Refractoriness. Soc. Exp. Biol. and Med. Proc., 34: 390. 1936.

(13) WERNER, S. C. Antibody Nature of Refractoriness to Injections of Hypophyseal Extracts Containing Thyrotropic Hormone. So. Exp. Biol. and Med. Proc., 34: 392. 1936.

(14) WESSON, L. G. A Fat Formation Under Abnormal Conditions from Carbohydrate by the Rat and its Relationship to a Possible New Dietary Factor. Jour. Biol. Chem., 73: 507. 1927.

MONTHLY VARIATIONS IN THE COMPOSITION OF MILK

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Results of analyses of milk produced by cows of the Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey breeds of dairy cattle and of Guernsey-Holstein crosses (the Bowlker Herd) have been reported in bulletins (1, 2) of the Illinois Agricultural Experiment Station.

The complete plan of the experimental work, including detailed descriptions of the method used to obtain samples and of procedures used in the analyses of the samples, and complete tabulations of the analytical results obtained, are included in those bulletins.

The purpose of the present publication is to study further the data presented in the two bulletins and to show the monthly variations in the composition of milk.

EXPERIMENTAL DATA

The samples of milk were taken usually at four- or five-week intervals during the lactation periods. All samples were three-day composites. Each sample is tabulated in the month in which it was obtained, and its composition is considered to represent the composition of the milk of the cow during the four- or five-week interval. The periods of time used for the samples were determined by dividing the time according to the weekly production records, considering the time represented by a sample to extend from the week-end nearest midway between it and the last preceding sample to the week-end nearest midway between it and the next succeeding sample.

TABLE 1

Monthly variations in the composition of milk
Ayrshire cows

Month	No. of cows	No. of samples	Percentages						Sp.G.
			Fat	Protein	Lactose	Ash	T.S.	S.N.F.	
Jan.	13	19	4.238	3.610	4.797	0.694	13.338	9.099	1.0321
Feb.	14	16	4.318	3.540	4.777	0.697	13.332	9.014	1.0322
Mar.	14	23	4.077	3.481	4.860	0.682	13.100	9.023	1.0327
Apr.	13	19	4.059	3.591	4.910	0.698	13.259	9.199	1.0332
May	11	16	3.908	3.386	4.879	0.682	12.856	8.948	1.0311
June	13	19	3.840	3.410	4.843	0.670	12.764	8.924	1.0322
July	10	13	3.840	3.373	4.829	0.665	12.707	8.867	1.0320
Aug.	11	15	3.810	3.464	4.869	0.659	12.804	8.994	1.0307
Sept.	12	16	4.113	3.564	4.655	0.664	12.997	8.884	1.0314
Oct.	12	17	4.134	3.564	4.713	0.683	13.096	8.962	1.0324
Nov.	11	19	3.989	3.565	4.691	0.674	12.918	8.929	1.0326
Dec.	10	16	4.044	3.531	4.841	0.676	13.092	9.048	1.0321

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TABLE 2

Monthly variations in the composition of milk
Brown Swiss cows

Month	No. of cows	No. of samples	Percentages						Sp.G.
			Fat	Protein	Lactose	Ash	T.S.	S.N.F.	
Jan.	17	41	4.067	3.649	5.054	0.731	13.503	9.436	1.0326
Feb.	17	37	4.006	3.683	4.892	0.726	13.308	9.302	1.0323
Mar.	16	33	3.874	3.612	4.930	0.724	13.141	9.267	1.0318
Apr.	16	31	3.818	3.494	4.966	0.719	13.031	9.214	1.0315
May	16	33	3.866	3.440	5.074	0.725	13.104	9.238	1.0311
June	16	32	3.700	3.337	5.106	0.718	12.888	9.188	1.0313
July	17	32	3.632	3.227	5.310	0.698	12.866	9.234	1.0306
Aug.	17	33	3.616	3.381	5.102	0.698	12.806	9.191	1.0300
Sept.	16	36	3.749	3.423	5.102	0.711	13.006	9.256	1.0306
Oct.	15	37	3.830	3.387	5.112	0.720	13.052	9.222	1.0326
Nov.	17	40	3.970	3.516	5.234	0.732	13.456	9.487	1.0325
Dec.	17	43	4.082	3.613	5.135	0.738	13.560	9.478	1.0330

As samples were taken during each week of every month, many samples do not represent milk which was produced wholly within the month in which the sample was obtained. For example, the period covered by a sample taken early in the month began during the latter half of the preceding month, while for one taken late in the month the period extended over into the first half of the succeeding month. However, as this overlapping is rather constant throughout the year, it is believed that it does not affect seriously the trends obtained for monthly variations in milk composition.

Tables 1 to 5 give, for the five breeds of dairy cows, the number of cows sampled each month, the number of samples analyzed, and the average composition based on the weight of milk produced.

TABLE 3

Monthly variations in the composition of milk
Guernsey cows

Month	No. of cows	No. of samples	Percentages						Sp.G.
			Fat	Protein	Lactose	Ash	T.S.	S.N.F.	
Jan.	16	34	5.384	4.089	4.858	0.764	15.046	9.712	1.0345
Feb.	14	23	5.160	3.931	5.037	0.752	14.880	9.720	1.0342
Mar.	15	31	5.151	4.010	5.019	0.750	14.930	9.779	1.0342
Apr.	12	22	5.056	3.773	5.055	0.740	14.624	9.568	1.0340
May	15	30	4.938	3.859	5.054	0.735	14.586	9.648	1.0333
June	15	28	4.698	3.660	5.044	0.719	14.120	9.422	1.0330
July	14	25	4.696	3.675	5.011	0.714	14.095	9.399	1.0325
Aug.	14	26	4.811	3.680	4.987	0.709	14.186	9.375	1.0323
Sept.	14	31	4.809	3.851	4.914	0.713	14.286	9.477	1.0333
Oct.	13	23	5.261	4.050	4.848	0.738	14.896	9.635	1.0340
Nov.	12	22	5.327	4.227	4.845	0.755	15.152	9.826	1.0350
Dec.	15	26	5.335	4.046	4.865	0.753	14.998	9.664	1.0342

TABLE 4

Monthly variations in the composition of milk
Holstein cows

Month	No. of cows	No. of samples	Percentages						Sp.G.
			Fat	Protein	Lactose	Ash	T.S.	S.N.F.	
Jan.	17	30	3.447	3.332	4.804	0.682	12.265	8.818	1.0326
Feb.	14	22	3.386	3.347	4.799	0.697	12.229	8.843	1.0327
Mar.	17	28	3.334	3.245	4.937	0.672	12.188	8.853	1.0324
Apr.	15	20	3.324	3.267	4.922	0.675	12.189	8.864	1.0327
May	18	25	3.329	3.229	4.919	0.684	12.162	8.833	1.0322
June	17	24	3.347	3.226	4.964	0.664	12.201	8.854	1.0319
July	15	21	3.362	3.309	4.896	0.673	12.240	8.878	1.0317
Aug.	13	16	3.311	3.285	4.977	0.656	12.228	8.917	1.0316
Sept.	11	17	3.530	3.406	4.995	0.678	12.614	9.079	1.0322
Oct.	16	20	3.523	3.482	4.808	0.673	12.485	8.963	1.0323
Nov.	14	22	3.622	3.333	4.821	0.662	12.440	8.818	1.0326
Dec.	16	23	3.392	3.210	4.854	0.680	12.136	8.744	1.0320

Table 6 gives similar information for the milk of the Guernsey-Holstein crossbred cows.

Table 7 summarizes the data which are included in tables 1 to 6.

DISCUSSION OF THE TABULATED DATA

The milk of the Ayrshire cows (table 1) varies from a high of 4.32[1] per cent of fat in February to a low of 3.81 per cent in August. The protein varies from 3.61 per cent in January to 3.37 per cent in July. The trend of the protein content follows that of the fat rather closely.

The lactose varies from 4.91 per cent in April to 4.66 in September. It

TABLE 5

Monthly variations in the composition of milk
Jersey cows

Month	No. of cows	No. of samples	Percentages						Sp.G.
			Fat	Protein	Lactose	Ash	T.S.	S.N.F.	
Jan.	14	21	5.293	3.910	4.875	0.710	14.786	9.493	1.0332
Feb.	13	17	5.134	3.870	4.997	0.726	14.727	9.594	1.0336
Mar.	14	21	5.100	3.767	5.011	0.698	14.575	9.475	1.0336
Apr.	12	14	5.297	3.810	5.094	0.716	14.918	9.621	1.0343
May	15	23	5.101	3.677	5.051	0.695	14.525	9.424	1.0327
June	12	19	4.866	3.590	5.080	0.684	14.220	9.354	1.0326
July	11	15	4.809	3.748	4.984	0.665	14.094	9.285	1.0322
Aug.	12	15	4.653	3.668	5.111	0.683	14.119	9.455	1.0328
Sept.	10	14	5.098	3.890	5.023	0.680	14.690	9.592	1.0333
Oct.	10	13	5.458	3.983	4.935	0.711	15.087	9.628	1.0344
Nov.	10	12	4.956	3.968	4.883	0.714	14.524	9.568	1.0344
Dec.	10	15	4.877	3.805	4.976	0.714	14.372	9.495	1.0341

[1] In the discussion all percentage values are given to the nearest second decimal.

TABLE 6

Monthly variations in the composition of milk
Guernsey-Holstein cross-bred cows
(Bowlker herd)

Month	No. of cows	No. of samples	Percentages						Sp.G.
			Fat	Protein	Lactose	Ash	T.S.	S.N.F.	
Jan.	53	82	4.448	3.710	4.810	0.739	13.708	9.259	1.0335
Feb.	55	84	4.287	3.589	4.895	0.720	13.489	9.202	1.0332
Mar.	58	92	4.231	3.543	5.021	0.722	13.516	9.284	1.0329
Apr.	60	104	4.147	3.563	5.043	0.720	13.473	9.326	1.0330
May	57	81	4.149	3.618	5.062	0.721	13.550	9.401	1.0326
June	51	79	4.009	3.535	5.020	0.710	13.275	9.266	1.0327
July	56	89	4.018	3.556	4.926	0.704	13.204	9.186	1.0319
Aug.	49	68	4.022	3.700	4.945	0.707	13.374	9.352	1.0320
Sept.	46	62	4.191	3.744	4.876	0.704	13.516	9.324	1.0329
Oct.	46	72	4.456	3.834	4.892	0.721	13.903	9.448	1.0334
Nov.	55	92	4.472	3.822	4.743	0.730	13.767	9.296	1.0336
Dec.	60	97	4.514	3.763	4.835	0.728	13.839	9.326	1.0332

TABLE 7

Monthly variations in the composition of milk
All samples

Month	No. of cows	No. of samples	Percentages						Sp.G.
			Fat	Protein	Lactose	Ash	T.S.	S.N.F.	
Jan.	130	227	4.314	3.669	4.867	0.723	13.567	9.259	1.0331
Feb.	127	199	4.221	3.616	4.891	0.719	13.447	9.226	1.0330
Mar.	134	228	4.158	3.561	4.976	0.711	13.405	9.247	1.0328
Apr.	128	210	4.104	3.542	5.005	0.713	13.372	9.267	1.0328
May	132	208	4.104	3.531	5.042	0.712	13.372	9.267	1.0322
June	124	201	3.961	3.448	5.019	0.698	13.132	9.171	1.0323
July	123	195	3.951	3.461	5.015	0.695	13.116	9.164	1.0317
Aug.	116	173	3.949	3.541	5.000	0.692	13.184	9.235	1.0315
Sept.	109	176	4.100	3.615	4.958	0.699	13.379	9.278	1.0321
Oct.	112	182	4.240	3.663	4.920	0.711	13.534	9.294	1.0330
Nov.	119	207	4.266	3.687	4.881	0.715	13.550	9.284	1.0332
Dec.	128	220	4.298	3.650	4.921	0.720	13.586	9.289	1.0331

TABLE 8

Differences between the highest and lowest monthly values

Breed	Percentages						Sp.G.
	Fat	Protein	Lactose	Ash	T.S.	S.N.F.	
Ayrshire	0.508	0.237	0.255	0.039	0.631	0.332	0.0025
Brown Swiss	0.466	0.456	0.418	0.040	0.754	0.299	0.0030
Guernsey	0.688	0.567	0.210	0.055	1.057	0.451	0.0027
Holstein	0.311	0.272	0.196	0.041	0.478	0.335	0.0011
Jersey	0.805	0.393	0.236	0.061	0.993	0.343	0.0022
Crosses	0.505	0.299	0.319	0.035	0.699	0.262	0.0017
All samples	0.365	0.239	0.175	0.031	0.470	0.130	0.0017

does not vary directly with the fat, but the tendency is for the higher lactose percentages to accompany the lower percentages of fat.

The ash content varies from 0.70 per cent in April to 0.66 per cent in August and follows closely with the percentage of fat.

The trends in total solids, solids-not-fat and specific gravity are similar to that of the fat.

The trends in monthly variation in composition of the milk of the other breeds and of all samples taken together (tables 2 to 7 inclusive) are similar to those for the milk of Ayrshire cows, although the highest and lowest values for the different breeds may not occur in the same months.

Table 8, which gives the differences between the highest and lowest values for each breed and for all samples, shows the comparatively greater variations in fat and total solids for Guernsey and Jersey milk. The variations in protein and lactose are less pronounced than that of the fat, and the solids-not-fat vary much less than the total solids.

SUMMARY

The average values for the composition of 2,426 milk samples, based upon the weight of milk produced, are tabulated according to breed of cow and month in which the samples were taken. The samples were distributed as follows: 208 samples of Ayrshire milk, 428 samples of Brown Swiss milk, 321 samples of Guernsey milk, 268 samples of Holstein milk, 199 samples of Jersey milk and 1,002 samples of milk from Guernsey-Holstein crossbred cows (the Bowlker Herd).

On account of the number of samples included and the distribution of the samples through the lactation periods of individual cows of the different breeds, the author believes that the variations shown fairly represent the milk of each breed of cows studied and that the variations shown for all samples taken together are similarly representative of the mixed milk of large numbers of cows of all breeds.

REFERENCES

(1) OVERMAN, O. R., GARRETT, O. F., WRIGHT, K. E., AND SANMANN, F. P. Composition of Milk of Brown Swiss Cows. With summary of data on the composition of milk from cows of other breeds. Ill. Agr. Expt. Sta. Bul. 457: 575–623. 1939.

(2) OVERMAN, O. R., SANMANN, F. P., AND WRIGHT, K. E. Studies of the Composition of Milk. Ill. Agr. Expt. Sta. Bul. 325: 51–174. 1929.

RELATION OF NUTRITION TO GROWTH AND BREEDING PERFORMANCE IN DAIRY BULLS. I. ALFALFA HAY RATIONS*

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Reproductive failure in cattle, either permanent or temporary, is one of the most serious problems confronting dairy herd owners. The dairy cow gives the largest amount of milk and butterfat on a life-time basis when she calves approximately once each year. Failure to conceive when bred may delay calving for several months at a distinct economic loss. In the case of a heifer, an even greater loss is caused by failure to conceive as she must be maintained additional months without any financial return.

Poor reproductive performance in a dairy herd may be caused by one or more factors, as discussed in an earlier report (9). The fault may lie with either the herd sire or the females. It may be due to the lack of adequate nutrition, to poor management practices, to disease conditions, to the inheritance of low fertility factors, or to physiological disturbances of the endocrine gland system, in turn affected by nutrition, heredity, or disease.

The studies of Williams and Savage (14), Donham, Simms, and Shaw (3), Brooke (1), Herman and Swanson (8), and others clearly show the variation in breeding efficiency of herd sires. The cause of poor breeding performance in bulls has not in most cases been definitely determined. The literature gives very little information on controlled feeding experiments dealing with fertility in the bull.

The feeding of dairy cattle on rations consisting solely, or almost entirely, of alfalfa hay for many months of the year is characteristic of many areas in the western states (10). In a survey (1) of twenty-five dairy herds, mostly Bang's disease free, in the alfalfa hay regions of Oregon, eighteen herds were reported having "difficult breeding." Microscopic examination of the semen of bulls in many herds reporting difficult breeding has given indications that, in many cases, the herd sires may be at fault. In many herds in the alfalfa hay regions the herd sire is maintained on alfalfa hay as the only feed, or alfalfa hay supplemented with limited grain, whereas the females have access to pasture for about 6 months each year.

Cunningham and Hopkirk (2) induced sterility in male rats by feeding very high protein diets (65–82 per cent) and by feeding a diet containing 15–18 per cent protein in which the protein was chiefly derived from maize or maize and gelatine. The sterility noted in mating tests was confirmed by

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semen motility and density studies, by morphological studies of spermatozoa and by histological examination of the testes. Degeneration of the testes followed a progressive pattern, becoming more marked the longer the rats continued on the diets. No reason is advanced to explain the destruction of germinal epithelium on the high protein diets thought to supply all of the dietary essentials, and on which good growth was obtained. The results on the rations containing mostly maize or maize and gelatin protein were thought to be due to the amino acid deficiencies characteristic of these foods. Male rats were found to be more susceptible to sterility due to excess of protein or deficient quality of protein than females.

Webster (13) in New Zealand has reported a high incidence of sterility and abnormal spermatozoa in bulls maintained on pastures in certain districts where the crude protein of the grass may be as high as 35 per cent of the dry matter. While the unlimited feeding of good alfalfa hay (14–15 per cent crude protein) alone supplies about 75 per cent more digestible protein than required by growing dairy animals after about 8 months of age, this excess protein is usually not regarded as harmful.

Smirnov-Ugrjumov and Laptev (12) have reported that the addition of proteins, especially animal proteins as supplied by blood meal and skim milk, to the rations of bulls caused a marked improvement in sperm concentration and resistance, the effect being on spermatogenesis as it was not evident before 17 to 37 days' feeding. Dry rations of hay and concentrates were less favorable for sperm production than those containing green feed, but feeding grass only caused an immediate decrease in sexual activity and amount of ejaculate while the effect on sperm concentration and resistance was noticeable 17 to 37 days later.

Young dairy animals will usually eat sufficient alfalfa hay to provide adequate total digestible nutrients to meet their normal growth requirements after about 7 months of age. Alfalfa hay alone may not supply sufficient phosphorus to maintain normal blood phosphorus values (6, 10). In 1938 the Oregon Agricultural Experiment Station began a series of investigations to study the importance of various nutritional factors that may cause low fertility or sterility in dairy bulls. This report presents the results of maintaining a group of bulls after 5 to 7 months of age on a ration of alfalfa hay, salt and disodium phosphate as compared to a second group fed the same ration supplemented with limited amounts of skim milk powder and oat groats. When this project was planned, not as much was known about the relation of protein quality to the nutritional well-being of ruminants as at present. The deficiency of alfalfa protein in sulphur-containing amino acids for normal growth and lactation in rats had been denomstrated (7, 15). It was hoped that this study would indicate the possible relationship between the amount and quality of protein in the ration, and the growth and fertility of bulls maintained thereon.

EXPERIMENTAL PROCEDURE AND RESULTS

Six purebred bull calves, three each of Jerseys and Holsteins, were selected for the experiment shortly after birth. Two Jerseys, 137B and 148B, and two Holsteins, 356B and 322B, were designated as Group A and were fed alfalfa hay, salt, disodium phosphate and potassium iodide solution after about 7 months of age. Two calves, Jersey, 143B, and Holstein, 354B, were placed in Group B and were fed the Group A ration supplemented with small amounts of high quality concentrates.

All calves received whole milk or skim milk with added fat and vitamin A until about 30 days of age, followed by skim milk for various periods. A grain supplement of ground oats or rolled oat groats was given in increasing amounts up to 3 pounds daily until 7 months of age. Good quality alfalfa hay, averaging 14.7 per cent crude protein, was fed in as large amounts as the calves would consume. The calves had free access to disodium phosphate, salt, and water at all times, and a potassium iodide solution was fed weekly.

The total feed consumption records of the individual calves from birth to 2 years of age are summarized in table 1. Calves 148B, 356B, and 143B received limited whole milk followed by skim milk, to which was added approximately 2 per cent each of salmon oil and cocoanut fat, the former to provide vitamin A.

The ages at which the individual calves in Group A were limited to alfalfa hay, supplemented only with potassium iodide, disodium phosphate, and salt are given in table 1. It will be noted that 148B received only alfalfa hay and minerals after 153 days, whereas the other calves were about 7 months old before the oat supplement was discontinued, and 322B was 7 months old before skim milk powder was discontinued.

The daily intake of alfalfa hay at 7 months of age was about 10 pounds daily, with the amount consumed increasing fairly rapidly so that the daily consumption at 24 months was about 22 pounds for the Jerseys and 27 pounds for the Holsteins.

Calf 322B showed poor coordination and stiffness when walking and lacked appetite at 3½ months of age. He was consuming a daily ration of 2 to 3 pounds of alfalfa hay and one pound each of skim milk powder and oat groats at the time, which was in early March. The inorganic blood phosphorus value was found to be about 50 per cent of normal for his age (6), whereas the other calves, which were 10 to 12 months old, showed almost normal values. Calf 322B was fed 10 cc. of cod liver oil daily for one week, which with increasing hay consumption caused him to grow normally.

Jersey bulls, 143B and 137B, went off feed at 11½ and 14 months of age, respectively, and it was necessary to feed skim milk for about a month, after which they again began to eat alfalfa hay and to grow. The average daily feed intake from two years until disposed of, as well as the age and weight at disposal, is given in table 2.

TABLE 1

Feed record and growth data (birth to two years of age)

	Group A				Group B	
	137B	148B	356B	322B	143B	354B
Whole milk, lbs.	168	81	76	295	42	352
Remade milk, 4% fat, lbs.*	.....	52	116	.....	162	.....
Skim milk, lbs.	1,522†	1,373‡	1,422	513	1,012	1,568
Ground oats, lbs.	108	144	145	.....	106	189
Oat groats, lbs.	69	137	286	275	749	694
Skim milk powder, lbs.	.....	.....	.....	236	559	539
Alfalfa hay, lbs.	9,101	10,237	12,223	11,604	9,221	12,208
Disodium phosphate, lbs.	26	27	16	36	26	16
Nutrients consumed, lbs.						
Crude protein	1,267	1,439	1,721	1,641	1,603	1,961
Digestible protein	914	1,041	1,231	1,179	1,242	1,490
Crude fat	162	185	223	211	211	270
Total digestible nutrients	4,825	5,494	6,494	6,429	6,043	7,533
Calcium	118	133	146	147	128	166
Phosphorus	23	27	28	30	31	37
Age off milk, days	160	137	172	217	.....	.....
Age off oats or oat groats, days	220	153	210	217	.....	.....
Birth weight, lbs.	61	71	93	106	58	98
Weight at 24 months, lbs.	830	920	1,295	1,260	949	1,469
Gain—birth to 24 months, lbs.	769	849	1,202	1,154	891	1,371
Average daily gain, lbs.	1.05	1.16	1.65	1.58	1.22	1.87
Total digestible nutrients per pound gain	6.27	6.47	5.40	5.57	6.78	5.49

* Equal parts salmon oil and cocoanut oil.
† Includes 510 lbs. fed between ages of 420 and 455 days when bull was off feed.
‡ Includes 375 lbs. fed between 344 and 370 days when bull was off feed.

The growth of the bulls is indicated by the weight curves shown in figure 1. It will be noted that Holstein bull, 354B, and Jersey Bull, 143 B, each of which received alfalfa hay supplemented with one pound each of oats or oat groats and skim milk powder after about 7 months of age, grew at a normal rate. Group A bulls, fed alfalfa hay with mineral supplements after 5 to 7 months, grew at a 10 to 15 per cent below normal rate between 7 and 24 months. The gain in weight of the individual bulls is definitely related to the total digestible nutrient intake as shown in table 1.

While Group B animals made considerably larger gains in weight, their efficiency of feed utilization, as measured by the calculated pounds of total

TABLE 2

Feed and other records (two years of age until disposal)

	Group A				Group B	
	137B	148B	356B	322B	143B	354B
Age at disposal, months	41.4	32.4	38.6	31.9	39.0	40.8
Alfalfa hay, average daily lbs.	26	26	34	28	24	32
Skim milk powder, average daily lbs.	.....	.....	.....	.....	1	1
Oat groats, average daily lbs.	.....	.....	.....	.....	1	1
Weight at disposal, lbs.	1,110	1,030	1,735	1,420	1,145	1,795

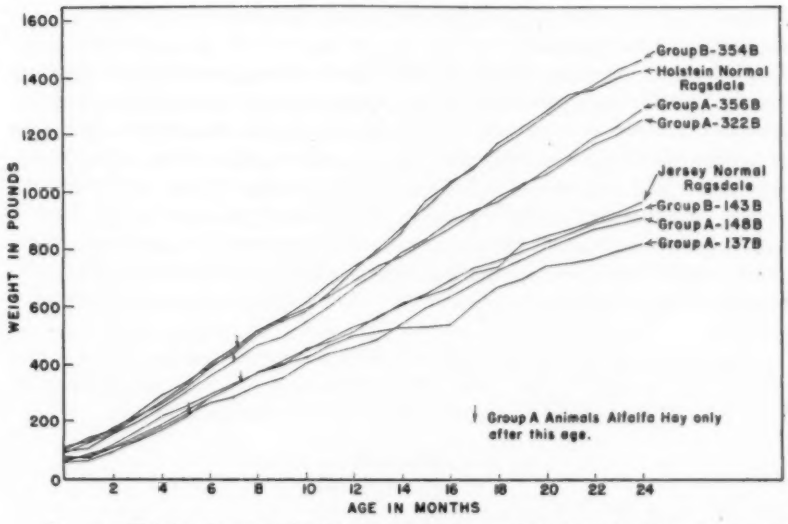

FIG. 1. Weight changes of individual bulls by months from birth to 24 months compared to Ragsdale normal.

digestible nutrients per pound of gain, did not average quite as high as Group A animals, the figures being 6.14 and 5.93, respectively. The Holstein bulls made more efficient use of the total digestible nutrients supplied than did the Jerseys, the former requiring an average of 5.49 pounds per pound of gain and the Jerseys 6.51 pounds.

The skeletal growth of the bulls as measured by height at withers to a lesser degree paralleled the weight changes, as is shown by comparing the figures in table 3 at 6 and 24 months of age with the weight curves in figure 1.

The age and weight of the bulls at disposal is indicated in table 2. As they became older, the bulls in Group A approached Group B animals in body weight. However, at all times after about 8 months of age Group B

TABLE 3

Growth in height

	Height at withers, cm.		Per cent normal	
	6 mo.	24 mo.	6 mo.	24 mo.
Group A				
137B	96.0	124.6	101.6	97.5
148B	92.1	124.4	97.5	97.3
356B	98.7	140.6	95.9	99.0
322B	103.5	140.8	100.6	99.2
Group B				
143B	94.8	130.6	100.3	102.2
354B	100.6	140.6	97.8	99.0

animals were more thrifty in appearance and showed better condition, particularly of hide and hair.

Collections of semen by use of an artificial vagina were made at approximately monthly intervals from one until two years of age, and then less frequently. Motility ratings were made either at the time the sample was taken or within 20 minutes afterwards. Sperm counts were made, using hemacytometers and pipettes used for erythrocyte counts, with 3 per cent NaCl as the diluting fluid. Hydrogen ion determinations were made with a potentiometer, using the glass and calomel electrodes. An ordinary blood smear method was used in making semen smears, which were stained by a modified eosin-hematoxylin method (5) after being fixed with Schaudinn's solution.

The results of the semen examinations are summarized in table 4. The average initial motility was obtained by allowing 4 for excellent, 3 for good, 2 for fair, 1 for poor, and 0 for no motility in the various samples.

TABLE 4

Semen examinations

	Number of samples	Initial motility, average	Volume, cc., average	Concentration per cmm./1,000, average	Abnormal forms, %, average	Original pH range
Group A						
137B	21	3.28	3.88	1,253	16.5	6.28–7.42
148B	18	2.56	3.68	1,136	12.0	6.61–7.51
356B	22	3.14	6.33	837	10.6	6.49–6.79
322B	12	3.33	6.44	634	5.9	6.30–6.92
Group B						
143B	26	3.54	3.70	1,360	20.0	6.31–6.71
354B	24	3.50	6.40	862	12.1	6.45–7.17

The 123 semen samples represented in table 4 showed considerable variation as obtained at different times from the 6 bulls. The average initial motility was rated excellent for 52 per cent of the samples in Group A as compared with 72 per cent of the samples in Group B. Bull 148B, subjected to the most restricted feeding as shown in table 1, showed excellent motility in only one-third of the samples and a like number with poor or no motility.

Sperm counts of the individual samples showed wide variations, the lowest individual value being 250,000 per cmm. for 356B and the highest 2,380,000 for 143B. The Jersey bulls used in this study produced a small amount of semen with greater sperm concentration and more abnormal forms as compared to the Holsteins. Only four samples in each of the two groups showed more than 30 per cent abnormal spermatozoa, and three of these in each group were collected from 137B and 143B.

A study of the type of abnormal forms of spermatozoa found in the individual samples showed that tail deformities accounted for 87, 89, and 93 per cent of these with the Jersey bulls 137B, 148B, and 143B respectively,

whereas with the Holstein bulls 356B, 322B, and 354B the tail deformities were respectively 56, 16, and 84 per cent of the total. The other types of abnormal spermatozoa noted were anterior enlargements, posterior enlargements, and narrow bases. These were found in relatively small and about equally distributed numbers.

While an occasional semen sample showed high or low pH values, more than 70 per cent of the values for semen with excellent motility fell between 6.4 and 6.8. Semen of low motility was often alkaline.

The semen studies gave some indication of improvement in motility and concentration from supplementing an alfalfa hay ration with oats or oat groats and skim milk powder. However, the bulls fed the supplemented ration showed a higher percentage of abnormal forms.

The variation in sperm motility, concentration, and per cent of abnormal sperm with age is given in table 5.

TABLE 5

Variation in semen quality with age of bulls

	Number of samples	Initial motility, average	Concentration per cmm./1,000, average	Abnormal spermatozoa, %, average
Group A				
Under 18 mo.	23	2.39	956	15.4
From 18–30 mo.	37	3.49	1,051	11.1
Over 30 mo.	13	3.08	662	4.2
Group B				
Under 18 mo.	19	3.21	1,269	21.4
From 18–30 mo.	24	3.88	1,165	11.1
Over 30 mo.	7	3.29	776	19.1

The bulls in Group A fed alfalfa hay alone produced sperm of relatively low motility before 18 months of age. This is probably a reflection of their greater immaturity compared to Group B animals, as indicated by the growth curves in figure 1.

A study of semen samples from 354B in Group B, collected daily for 31 consecutive days when about 2 years of age, has been reported elsewhere (4). These samples are not included in the averages in tables 4 and 5. While there was considerable daily variation in semen characteristics of these 31 samples, the ones with the largest sperm counts showed the greatest motility.

Samples with original pH values around 6.6 usually showed better motility than samples around 7.0 or on the alkaline side. The average initial motility of the 31 consecutive day samples was 3.16 compared to 3.50 for 24 samples taken from 12.8 to 40.9 months, as reported in table 4.

Each of the bulls had limited service to females in the college herd and, with the exception of 322B, were used in service in the herds of cooperating dairymen for a 9- to 12-month period when from 2 to 3½ years of age. The results of service are given in table 6.

A study of table 6 shows that all bulls proved fertile and had a low number of average services per conception. Of the cows sold or re-bred to other bulls, five in Group A and 9 in Group B were sold as dairy animals without pregnancy definitely determined, and three in Group A and 2 in Group B were sold as non-breeders. Of the five cows bred to 322B in the college herd, the conception was with a cow on fifth service. The four cows not conceiving were bred once each at the 1st, 3rd, 5th, and 6th services, with two cows going to the butcher as non-breeders after 7 and 8 services, and two calving after the 4th and 6th services.

TABLE 6

Service records

Group Bull no.	Age during service	Cows bred	Cows conceived	Ave. services per conception	Normal calves	Cows sold or rebred other bulls	
	months	*no.*	*no.*	*no.*	*no.*	*no.*	*ave. services*
Group A							
137B	29.2–40.0	22	14	1.57	13	8	3.13
148B	24.7–34.1	21	21	1.19	21	0	.....
356B	27.2–36.3	17	11	1.09	11	6	2.33
322B	26.0–29.8	5	1	1.00	1	4	6.25
Total and average		65	48	1.27	46	18	3.56
Group B							
143B	27.4–36.9	20	13	1.69	9	7	1.71
354B	28.3–40.3	38	28	1.43	25	10	1.80
Total and average		58	41	1.54	34	17	1.76

SUMMARY

Four bulls, two each purebred Jersey and Holstein, fed a basal ration of alfalfa hay containing between 14 and 15 per cent crude protein, disodium phosphate, salt, and potassium iodide solution after 5 to 7 months of age and until disposal at 32 to 41 months of age, grew at a 10 to 15 per cent below normal rate until about 2 years of age. Semen samples showed more desirable characteristics after 18 months of age than before. All bulls proved fertile on service at from 24 to 40 months of age.

Two bulls, one each purebred Jersey and Holstein, fed the basal ration plus a daily supplement of one pound each of skim milk powder and oat groats after 7 months of age, grew at a normal rate. They produced semen with more desirable characteristics, especially up to 18 months of age, as compared with the bulls fed the basal ration. The bulls proved fertile on service.

Jersey bulls produced a smaller volume of semen with higher sperm concentration compared to Holsteins. The total sperm per ejaculation, averaging for the individual animals between 4 and 5½ billion, the average initial motility and the original pH of the semen was not greatly different for the two breeds. A higher percentage of abnormal forms, particularly tail de-

formities, was found in semen from Jersey bulls, but the number was not usually high enough to characterize the semen as of poor quality.

Sixty-seven samples of semen with excellent original motility, disregarding breed and age, measured 5.4 cc., showed a sperm concentration of 1,120,000 per cmm., had 11 per cent abnormal spermatozoa and in 70 per cent of the samples gave initial pH readings between 6.40 and 6.80.

CONCLUSIONS

Dairy bulls limited to a basal ration of good alfalfa hay supplemented with salt, phosphorus, and iodine after about 7 months of age grew at a 10 to 15 per cent below normal rate until about 2 years of age, produced good quality semen after 18 months of age and were fertile on service between 2 and 3½ years of age.

Bulls fed the basal ration supplemented with one pound each of skim milk powder and oat groats daily grew at a normal rate, produced good quality semen after 12 months of age, and were fertile on service.

It is believed that the faster growth, earlier maturity and better condition of the bulls fed the supplement was due to the greater energy intake rather than to the increased amount or any improvement in quality of protein.

REFERENCES

(1) Brooke, R. E. Sterility in Dairy Cattle. Master's Thesis, Libr., Oreg. State Col. 1931.

(2) Cunningham, I. J., and Hopkirk, C. S. M. Dietary Protein in Relation to Sterility. New Zeal. Jour. Sci. and Technol., **17**: 420–432. 1935.

(3) Donham, C. R., Simms, B. T., and Shaw, J. N. Fertility Studies in the Bull. Amer. Vet. Med. Assoc. Jour., **78**: 658–680. 1931.

(4) Dougherty, R. W., and Ewalt, H. P. Semen Studies in the Bull. Amer. Jour. Vet. Res., **2**: 419–426. 1941.

(5) Gradwohl, R. B. H. Clin. Lab. Math. and Diag. C. V. Mosby Co., St. Louis. 2nd Ed., p. 710. 1938.

(6) Haag, J. R., and Jones, I. R. The Calcium and Inorganic Phosphorus Content of the Blood Plasma of Normal Dairy Cattle. Jour. Biol. Chem., **110**: 439–441. 1935.

(7) Haag, J. R. The Physiological Effect of Rations Restricted Principally or Solely to the Alfalfa Plant. II. Cystine as a Limiting Factor in the Nutritive Value of Alfalfa Proteins. Jour. Nutr., **4**: 363–370. 1931.

(8) Herman, H. A., and Swanson, E. W. Variations in Dairy Bull Semen with Respect to Its Use in Artificial Insemination. Mo. Agr. Expt. Sta. Res. Bul. 326. 1941.

(9) Jones, I. R., Dougherty, R. W., and Haag, J. R. Reproductive Performance in Dairy Cattle. Oreg. Agr. Expt. Sta. Bul. 395. 1941.

(10) Jones, I. R., Brandt, P. M., and Haag, J. R. Studies with Alfalfa Hay for Milk Production. Oreg. Agr. Expt. Sta. Bul. 328. 1935.

(11) Sherwood, D. H., and Dean, H. R. Feeding Alfalfa Hay Alone and with Concentrates to Dairy Cows. Oreg. Agr. Expt. Sta. Bul. 380. 1940.

(12) Smirnov-Ugrjumov, D. N., and Laptev, M. A. The Feeding of Stud Bulls during Mating Season. Probl. Zhirotn., No. **2**: 64–72.

(13) WEBSTER, W. M. Bovine Sterility in New Zealand. Austral. Vet. Jour., 8: 199–122. 1932.

(14) WILLIAMS, W. W., AND SAVAGE, A. Methods of Determining the Reproductive Health and Fertility of Bulls. Cornell Vet., 17: 374–385. 1927.

(15) WRIGHT, L. D., AND HAAG, J. R. The Lactation Promoting Effect of Cystine When Fed with Alfalfa Proteins. Jour. Nutr., 17: 263–268. 1939.

SEEPAGE LOSSES FROM A SILO*

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Seepage losses in making silage have long been a subject for speculation and are mentioned frequently in earlier silage investigations, but relatively few actual measurements of the extent of the loss have been made. The earliest quantitative record we have been able to find is that reported by Shaw *et al.* (4), who collected the juice from a 150-ton silo filled with corn in 1914 and again in 1915. The weights of juice collected varied from 2579 pounds the first year to 9495 pounds the second year. Nitrogen losses calculated as protein were 28.9 and 150.8 pounds respectively. Other constituents of the juice apparently were not determined. In 1923 Godden (2) reported on the composition of drainage liquor from silage made from a mixture of beans, peas, oats and tares in a 12-foot, 110-ton silo. His results indicated a material loss in nitrogenous compounds and minerals in the liquid. Recently workers at the New Jersey Experiment Station (1) have stated that losses from this source varied from 2.25 to 18 per cent of the ensiled material. Even more recently Otis (3) has published a detailed description (together with results of one year's work) of an experimental silo so constructed and fitted with special devices as to facilitate a comprehensive study of the problem.

EXPERIMENTAL

Our study of the problem was begun in 1938, and results for seven seasons are now available. The set-up consists of a 100-ton wooden stave silo with a concrete floor, which is graded to a central drain protected to permit adequate percolation. This drain leads by a cast iron 3″ pipe into an enclosed catch basin outside the silo. On a ledge in this chamber is set an automatic recorder similar in principle to a self-recording rain gauge (figure 1). This gauge is calibrated in place each season just before filling is started, and is read each morning as long as there is any considerable drip. The capacity of the double-action, tipping receptacle is approximately one liter, each round trip of the mechanism being recorded on a counter. As indicated in figure 1, representative sampling of the seepage liquid is assured by means of a small copper tube soldered to one side of the double bucket; on alternate tippings of the mechanism this tube leads off into a sample bottle a small aliquot of the liquid. For the most part the silage has been from mixed grasses and legumes, the grasses being predominant. In two seasons, however, there were much larger proportions of legumes, some lots being from nearly pure legume seedings.

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RESULTS

The average amount of seepage for the seven-year period is shown by the solid line in figure 2. Extremes are shown by the dotted line and the dot-dash line. These were in 1942 and 1944 respectively. Practically all of the seepage occurred during the first three weeks, most of it in the first week. The average total amount to the 48th day was 1707 liters, or 451 gallons. In some years the seepage stopped before that time; in all it had tapered off to an insignificant dribble by that time. The maximum daily amount recorded was 1208 liters on the second day after drainage had started in 1939.

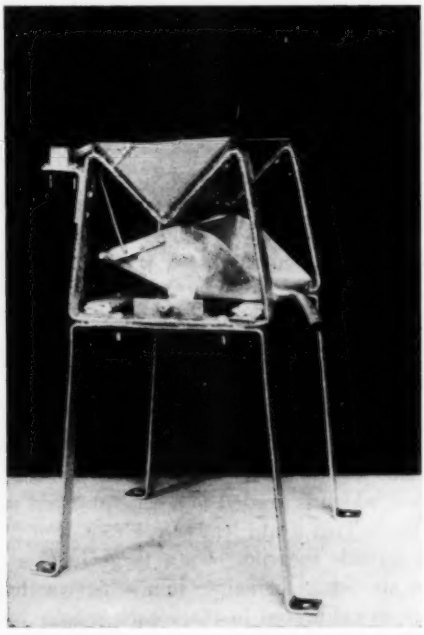

FIG. 1. Self-recording gauge used in these studies.

This excessive amount was never even remotely approached on any other day of any year; it is indicative of the extremely wet conditions sometimes noted in silo-filling operations, which should be avoided if at all possible. Such excessive amounts of seepage are a nuisance from the standpoint of both odor and attraction of flies; and yet if this excessive moisture were not allowed to drain off, it would be productive of a very undesirable grade of silage in the lower levels of the silo. Our experience over the years has finally led to the elimination of most of the seepage through wilting, use of less succulent crops, and addition of such absorbent preservatives as ground

wheat and ground corn. The minimum seasonal seepage has been attained this current year (1944) (see the dot-dash line in figure 2); the largest daily amount was 20 liters on the ninth day; the total for 43 days was approximately 294 liters. The maximum seasonal seepage was in 1942, when 3440 liters drained off from a rather succulent crop of legume silage, parts of which were nearly pure Ladino clover. The break in downward trend around the fourth to sixth day is presumably caused by increasing pressure as the silo filled.

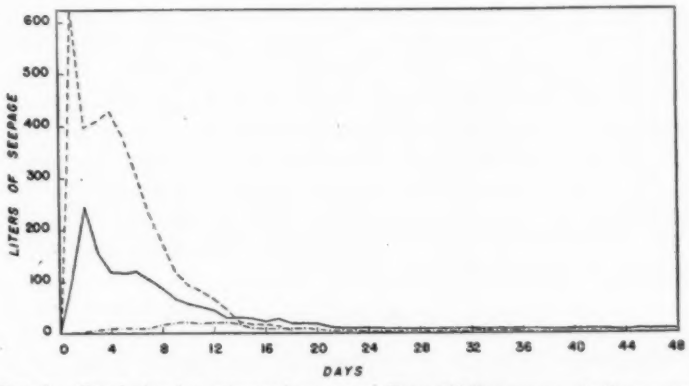

FIG. 2. Graph showing extent of seepage. The solid line represents the average for seven years; the dotted line shows the maximum seasonal amount and the dot-dash line the minimum. The zero point is the time when seepage began, which was usually 2 to 3 days after filling had been started.

A study has been made of the composition of the juice, quite complete in some years, less so in others, with the following results:

	Average (%)	Range (%)
Total solids	7.6	4.6 –10.0
Nitrogen	0.3 (1.9*)	0.25– 0.39
Ash	2.1	1.7 – 2.4
Ether extract	0.2	0.11– 0.34
Precipitable by alcohol	0.9	0.7 – 1.0
pH	4.3	4.0 – 4.7

* Protein equivalent.

Sugar was determined on two occasions. None was present the first time, and a value of 0.53 per cent total sugar expressed as invert sugar was obtained the second time. Two tests were made for carotene, but both were negative.

Calculation of Seepage Losses

By applying the figures for total solids in the seepage to the recorded losses of liquid, and relating the result to the total tonnage and average

moisture content of the silage stored, the following losses in dry matter are noted:

Average loss for 7 years	0.54%
Maximum loss—1942	1.08%
Minimum loss—1944	0.12%

In fairness it must be admitted that in certain seasons, notably 1939 and 1942, not all the seepage went through the gauge. Some of it leaked through the walls of the silo and over the edge of the concrete base; but supposing that as much, or even twice as much, was lost in this way as was recorded, the loss of dry matter under the least favorable conditions (1942) would be only about 3.25 per cent. This is a relatively small figure compared with losses commonly occurring from mold and rot at the top and sides of silos. Furthermore, the insignificant amount of seepage this year shows that with good management this need never be a source of more than very minor loss. From the nuisance standpoint it should be kept at a minimum, but since nutrient losses are so small, no attempt should be made to hold back the seepage. The improved quality of the silage when the juice is permitted to drain off will largely offset any small losses.

CONCLUSION

From a study of seepage measurements from a 100-ton silo over a period of seven years it is concluded that:

1. Seepage losses in silage are not as serious as those from other causes.
2. With good management they can be reduced to a very insignificant figure.

REFERENCES

(1) ANONYMOUS. N. J. Agr. Expt. Sta. Page 17. Report for 1940–41.
(2) GODDEN, W. Notes on the Drainage from Tower Silos. Jour. Agr. Sci. (England), 13: 462–466. 1923.
(3) OTIS, C. K. Methods of Moisture Drainage from Silos. Agr. Engin., 23: 321–323. 1942.
(4) SHAW, R. H., WRIGHT, P. A., AND DEYSHER, E. F. Nitrogen and Other Losses During the Ensiling of Corn. U. S. Dept. Agr. Bul. 953, 16 pages. 1921.

FINAL REPORT OF THE SUB-COMMITTEE ON THE DETERMINATION OF THE PERCENTAGE OF FAT IN BUTTERMILK, SKIM MILK AND WHEY

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The committee evaluated various centrifugal testing methods for estimation of the fat content of buttermilk, skim milk and whey. The Mojonnier test was used as a standard for comparison. The purpose was to find a satisfactory method for determining the fat content of these products. Selection of a testing method was based chiefly on simplicity and on agreement with the standard method.

With all centrifugal methods for natural buttermilk, skim milk and whey, as the fat content increased, there was a tendency for the fat test to read a higher percentage of the Mojonnier.

Testing methods employing amyl alcohol or fusel oil were unsatisfactory, apparently due to variability of the reagents.

While no one testing method agreed perfectly with the Mojonnier, some procedures conformed much better than others. The following tests gave the best results on the products and are recommended by the committee for adoption:

1. American Association (1); for buttermilk and skim milk and for whey from Cheddar, Blue, Edam and Swiss cheeses.
2. Minnesota (original reagent) (2); for whey from Cheddar, Swiss, Edam and Cottage cheeses.
3. Pennsylvania (3); for whey from Cheddar, Blue and Edam cheeses.

DIRECTIONS FOR THE AMERICAN ASSOCIATION METHOD

I. *Equipment*

Regular Babcock testing equipment and glassware, including 18-gm. 0.5% and 0.25% skim-milk test bottles.

II. *Reagents*

Normal butyl alcohol and sulphuric acid, specific gravity 1.82 to 1.83.

III. *Procedure*

1. Transfer to the test bottle 2 ml. N-butyl alcohol from a burette.
2. Add 9 ml. buttermilk, skim milk or whey and mix well.
3. Add 7 to 9 ml. sulphuric acid and mix well.
4. Centrifuge six minutes and add water to the base of the bottle neck.
5. Centrifuge two minutes and add water to bring the fat into the neck of the bottle.

6. Centrifuge two minutes and place the bottles in a water bath at 135° to 140° F. for 5 minutes.

7. Multiply the test by 2.

DIRECTIONS FOR THE ORIGINAL MINNESOTA METHOD

I. *Equipment*

Regular Babcock testing equipment and glassware, including 18-gm. 0.5% and 0.25% skim-milk test bottles.

II. *Reagents*

Minnesota test solution: 110 gm. sodium carbonate and 200 gm. sodium salicylate made to 1,000 ml. with distilled water. To this solution add 30 ml. 50% sodium hydroxide and 100 ml. normal butyl alcohol.

III. *Procedure*

1. Transfer to the test bottle, 9 ml. whey.

2. Add 10 ml. Minnesota reagent, shake thoroughly.

3. Digest in a water bath (160° to 178° F.) for six to seven minutes and shake several times during this interval.

4. Centrifuge five minutes and add water (135° to 140° F.) to bring the contents to the base of the neck.

5. Centrifuge two minutes and add water (135° to 140° F.) to bring the fat column into the graduated column of the neck.

6. Centrifuge one minute and place the tests in a water bath (135° to 140° F.) for five minutes.

7. Multiply the fat reading by 2.

DIRECTIONS FOR THE PENNSYLVANIA METHOD

I. *Equipment*

Regular Babcock equipment and glassware, including 0.5% and 0.25% skim-milk test bottles.

II. *Reagents*

Ammonium hydroxide, 28 to 29 per cent NH_3, normal butyl alcohol b.p. 117° Centrigrade, and diluted commercial sulphuric acid, specific gravity 1.72 to 1.74. The acid is diluted by adding 3½ parts, by volume, of commercial sulphuric acid, specific gravity 1.82 to 1.83, to 1 part of water.

III. *Procedure*

1. Pipette 17.5 ml. whey into a test bottle.

2. Add 2 ml. ammonium hydroxide from a burette and mix well.

3. Add 3 ml. butyl alcohol and mix thoroughly.

4. Add 17.5 ml. of the diluted sulphuric acid and mix until digestion is complete.

5. Centrifuge five minutes, add water (130° to 140° F.) to within ¼ inch of the neck.

6. Centrifuge two minutes, and add enough water (130° to 140° F.) to bring the fat within the graduated portion of the neck.

7. Centrifuge one minute. Place in water bath at 130° F. for five minutes and read the test directly.

REFERENCES

(1) MITCHELL, J. W. Churning Losses. Chicago Dairy Produce, 28, No. 31: 16–19. 1921.

(2) PETERSEN, W. E., AND HERREID, E. O. A New Method for Estimating the True Fat Content of Buttermilk. Minn. Agr. Expt. Sta., Tech. Bul. 63. 1929.

(3) SWOPE, W. D. The Pennsylvania Method for Determining the Percentage of Fat in Dairy Products. Penn. State Col. Agr. Expt. Sta. Bul. 412. 1941.

GENERAL APPEARANCE, GROWTH AND REPRODUCTION OF THYROIDECTOMIZED BOVINE[1]

A. A. SPIELMAN,* W. E. PETERSEN, J. B. FITCH, AND B. S. POMEROY
University of Minnesota, St. Paul, Minnesota

Few reports dealing with the effects of thyroidectomy upon the general appearance and growth of the bovine have come to the attention of the authors. Brody and Frankenbach (2) reported that thyroidectomy of a Jersey heifer at 54 days of age reduced mature body weight by over 50 per cent. Practically no weight gain was made after 18 months of age. The characteristic symptoms of hypothyroidism began to appear about a month after thyroidectomy. Andrews and Bullard (1) reported rapid fattening and good gains of steers during the first six weeks following partial thyroidectomy.

Only two reports have been found in the literature regarding the effects of hypothyroidism upon reproduction in the bovine. Petersen, Spielman, Pomeroy and Boyd (4) observed normal spermatogenesis in a sexually mature Jersey bull for 12 months following thyroidectomy, although libido was completely absent. Normal sexual behavior was restored by thyrotherapy. Brody and Frankenbach (2) reported complete absence of estrus in a mature Jersey heifer which had been thyroidectomized at 54 days of age. Subsequent replacement therapy resulted in normal estrus.

The paucity of information regarding the effects of thyroidectomy upon the bovine, especially pertaining to growth and reproduction, would seem to justify the present report.

The data in this report were accumulated during the course of our studies (7) on lactation in thyroidectomized bovine.

EXPERIMENTAL

Six thyroidectomized animals, one male and five females, were available for observation. The breed, sex, age and length of experimental period are given in table 1. Exploratory operations and autopsy examinations revealed incomplete removal of the thyroid from A15 and complete removal from the other experimental animals. Parathyroid tissue embedded in the thyroid was unavoidably removed: however, the accessory parathyroids were left intact. The amount of remaining functional parathyroid tissue was not determined.

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* Now Assistant Dairy Husbandman, Washington Agricultural Experiment Station.

[1] Taken from data presented in a thesis to the Graduate Faculty of the University of Minnesota by A. A. Spielman in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Scientific Journal Series Paper No. 2205, Minnesota Agricultural Experiment Station.

The experimental animals were maintained under practical herd management conditions. Body weight and skeletal height at the withers were measured monthly.

Semen samples were obtained by rectal manipulation of the ampullae and vas deferens. The presence, morphology and motility of the sperm was determined by microscopic examination. As a final test of fertility, normal and thyroidectomized cows were artificially inseminated.

Manual examination of the ovaries to determine ovulation was performed periodically by skilled veterinarians. Conception following artificial insemination was taken as proof of the production of fertile ova.

RESULTS AND DISCUSSION

Observations on behavior and general appearance. Although observations on 2009 were terminated by death 60 days after thyroidectomy, as shown in table 1, definite changes in appearance and behavior were evident

TABLE 1

Experimental animals

Animal No.	Breed	Sex	Age at thyroidectomy	History	Length of experimental period
2009	Jersey	F	34 days	Normal	60 days
A26	Holstein	F	13 months	Virgin	2 years
A23	Holstein	F	16 months	46th day, 1st gestation	2 years
E343	Jersey	M	15 months	Normal	2 years
A15	Holstein	F	36 months	12th day, 1st lactation	14 months
E294	Holstein	F	45 months	7th day, 2nd lactation	18 months

about 30 days following the operation. Diminished appetite, decreased activity, increased susceptibility to low temperatures, and enlargement of the paunch were observed. No gross evidence of pathology was found upon autopsy.

Visual symptoms of hypothyroidism in A26 and E343, thyroidectomized at 13 and 15 months of age respectively, were not apparent until about 2 months after thyroid removal. However, as shown in figure 7, chronic changes in appearance soon became quite distinct. The hair became progressively dry and brittle and the skin became thick and dry. The hind limbs were swollen and the movements slow and clumsy. The animals seemed to have difficulty in raising the feet and, in walking, shuffled along with a peculiar gait unlike that of a normal animal. On standing, the limbs were spread out so as to form a broad base of support. About four months after thyroidectomy, marked enlargement of the hock joints was observed. An excessive accumulation of synovial fluid in the joint capsule was found

upon autopsy. Marked intraperitoneal bloat, first observed about three months after thyroidectomy, persisted until death, and was found at autopsy to be due to fluid in the intraperitoneal cavity. Coincident with a subnormal body temperature, a decreased pulse rate was noted. Appetite decreased and constipation became chronic.

Thyroidectomy of E294 at the age of 45 months had very little effect on general body conformation and appearance except for an increased accumulation of fat. However, apathy, lethargy, increased susceptibility to cold and diminished reflexes were evident.

No post operative changes in general appearance or activity were noted in the case of the partially thyroidectomized cow, A15. As shown in table 1, A23 was completely thyroidectomized at 16 months of age and on the 46th day following conception. A23 failed to develop the characteristic symp-

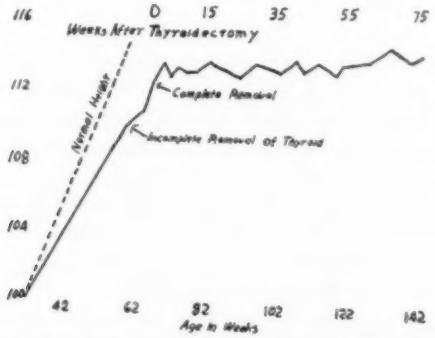

FIG. 1. Comparison of age curves of height at the withers of thyroidectomized Jersey male E343 and normal Jersey males.

toms of hypothyroidism observed in the other experimental animals. This observation will be discussed later in the report along with the growth behavior.

It is apparent that, if thyroidectomized bovine are kept alive, chronic changes in appearance and behavior do appear which may be described as bovine cretenism and myxedema. Similar symptoms of hypothyroidism, as reviewed by Koger and Turner (3), have been recorded for a great number of animals including the mouse, the guinea pig, the cat, the rabbit, the goat, the cow, and the monkey. Apparently the effects of hypothyroidism are similar in all animals.

Observations on growth. The data on growth were obtained on three thyroidectomized animals, E343, A26 and A23, and consist of measurements of body weight and height at withers. Growth data of E343 were accumulated for a period of 75 weeks after thyroidectomy. The effects of incomplete and complete thyroidectomy on height gain are shown in figure 1.

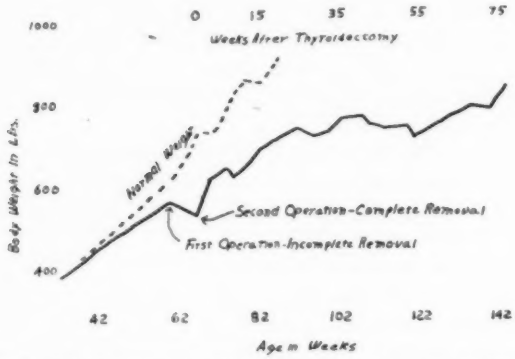

FIG. 2. Body weight curves of thyroidectomized Jersey male E343 and normal Jersey males.

Gain in height was slightly retarded for about five weeks after incomplete thyroid removal, followed by a period of normal gains. However, no significant increase in skeletal height occurred after complete thyroidectomy.

As shown in figure 2, there was a loss of body weight following incomplete thyroidectomy, although E343 continued to gain in skeletal height. Following complete thyroidectomy, weight gain was accelerated for about three weeks, after which the growth rate began to decline.

The effects of thyroidectomy on growth of A26 as shown in figures 3 and 4 were very similar to those observed for E343.

A23 was thyroidectomized on the 46th day of her first gestation period. Figures 5 and 6 show that growth was static for a period of about 20 weeks following thyroidectomy. However, coincident with the last ten weeks of

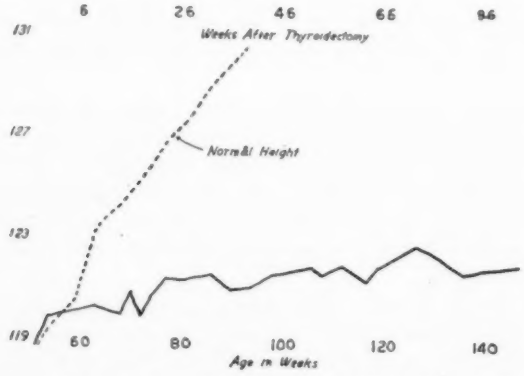

FIG. 3. Comparsion of age curves of height at the withers of thyroidectomized Holstein heifer A26 and normal Holstein females.

the gestation period, a sudden resumption of height and body weight gain occurred. Following parturition, growth again became static, and little change was noted until the latter part of the subsequent gestation period when growth was resumed. Although some of the increase in weight during pregnancy was due to the pregnant uterus and developing fetus, it was insufficient to account for the increase that occurred.

From the results observed, it seems evident that thyroidectomy of immature bovine is followed by a serious impairment of the normal growth processes. There is a complete stasis of height gain and severe retardation of body weight gain. These results confirm the report of Brody and Frankenbach (2). The results of incomplete thyroidectomy on growth were similar to those reported by Andrews and Bullard (1).

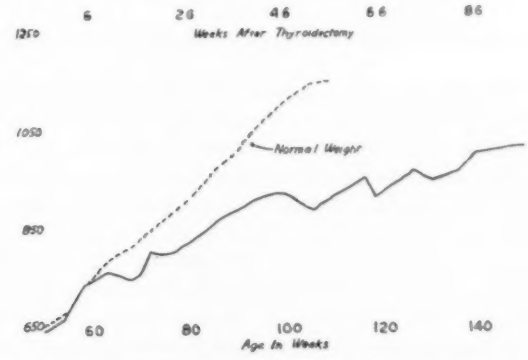

FIG. 4. Body weight curves of thyroidectomized Holstein heifer A26 and normal Holstein females.

The resumption of growth and the failure to develop the characteristic symptoms of hypothyroidism by a cow thyroidectomized during pregnancy is noteworthy. It appears that the thyroid deficiency of the mother was remedied, partially at least, either by diffusion of the thyroid hormone from the fetus into the maternal circulation, or by some other undefined factor related to the pregnant state. Similar observations on thyroidectomized goats have been reported by Reineke and Turner (5).

Observations on reproduction. The effects of complete thyroidectomy on sexual behavior, gametogenesis and fertility of sexually mature male and female bovine were studied.

Male. One thyroidectomized male, E343, was available for study. Observations on sexual behavior, spermatogenesis and thyrotherapy during the first 12 post-operative months have been published in detail previously by Petersen, Spielman, Pomeroy and Boyd (4), and will be summarized here. Complete inhibition of libido, but no apparent effect on spermatogenesis, was

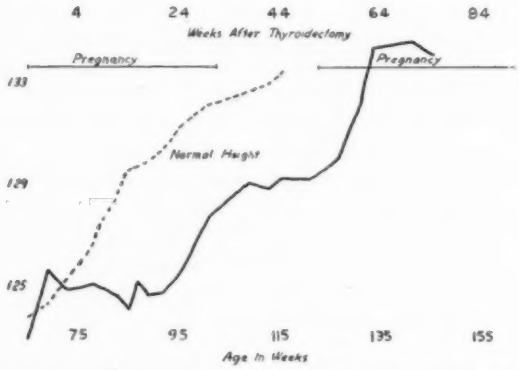

FIG. 5. Effects of pregnancy upon height gain of thyroidectomized Holstein heifer A23.

observed. Oral administration of fresh thyroid, dinitrophenol and testosterone propionate temporarily restored normal breeding behavior.

Subsequent data on fertility are presented in table 2.

Since this bull manifested no interest in cows in estrus, it was necessary to use artificial insemination. Semen was obtained by rectal manipulation of the ampullae and vas deferens. Of the eight cows artificially inseminated, three were normal, three were phosphorus deficient, and two were thyroidectomized. Five cows conceived to the first insemination, and three cows required two inseminations each. Normal semen was obtained for a period of several months following the last insemination. These data conclusively show that spermatogenesis continued after thyroidectomy even though libido was absent.

Female. Three completely thyroidectomized cows and one partially thy-

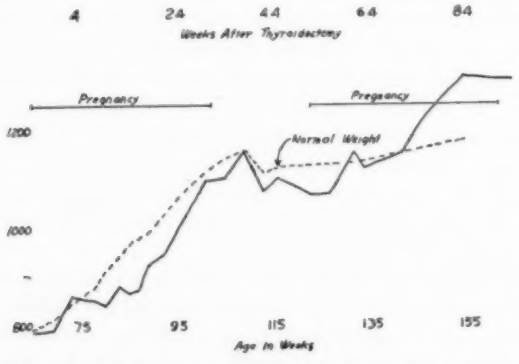

FIG. 6. Effects of pregnancy upon body weight gain of thyroidectomized Holstein heifer A23.

TABLE 2

Reproduction record of thyroidectomized male, E343

Days after thyroidectomy		Cow mated No.	Condition of cow	No. of insemi-nations	Results
Incom-plete	Com-plete				
394	84	E248	Phosphorus-deficient	2	Sold, definitely pregnant
401	91	E249	"	1	" " "
426	112	E245	"	1	" " "
490	176	A26	Thyroidectomized	1	Normal female calf, A46
517	203	A21	Normal	1	" " " , A50
535	221	A23	Thyroidectomized	2	" " " , A51
538	224	E294	"	2	Normal male calf, BA3
677	363	E376	Normal	1	Pregnant

roidectomized cow were available for observation. Table 3 presents data on the reproduction record of these cows.

A15, partially thyroidectomized 37 days after breeding, aborted a dead fetus on the 225th day of the gestation period. A negative reaction to the agglutination test for brucellosis was recorded. No satisfactory explanation is known. Simpson (6) considered it significant that among his pregnant thyroidectomized sheep no abortions were known to occur.

Thyroidectomy of A23, performed 45 days after conception, had no apparent effect on the duration of pregnancy. The gestation period was 272 days in length and terminated with the birth of a normal female calf.

Without exception, all of the completely thyroidectomized cows failed to manifest the normal physical signs of estrus. Absence of the normal physical expressions of estrus was confirmed by the complete lack of interest shown these cows by normal males. Oral administration of fresh thyroid to E294 elicited marked increases in milk and fat yield (7) and restored normal estral behavior. Thyrotherapy of A26 failed to evoke a response in either milk secretion (7) or estral behavior. In the case of A26, this may be partially ascribed to progressive impairment of the normal physiological

TABLE 3

Reproduction record of thyroidectomized females

Animal No.	Bred days after thyroidectomy	No. of insemi-nations	Male used	Gestation period	Progeny
				days	
A23	45*	1	Normal	272	Normal
A15	37†	1	"	225	Abortion
E294	161	2	Thyroidectomized	285	Normal
A26	242	1	"	279	"
A23	453	2	"	267	"

* Before thyroid removal.
† Incomplete thyroidectomy.

processes resulting in loss of ability to respond to thyroid therapy, as 546 days had elapsed since thyroidectomy.

Although estrus was absent, rectal examination of the ovaries indicated that ovulation occurred at regular intervals. Definite proof that ovulation occurred and that it was accurately determined by manual examination is shown by the fact that, of the four anoestrus conceptions, two required one insemination each and the other two required two inseminations each.

As shown in table 3, artificial insemination of E294, A26 and A23, 161, 242, and 453 days after complete thyroidectomy, with semen from a completely thyroidectomized male, resulted in the birth of three normal off-

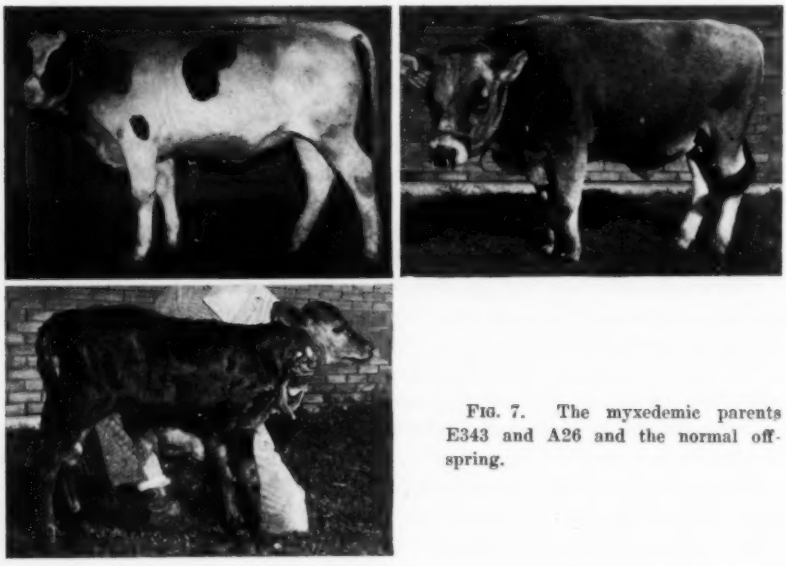

FIG. 7. The myxedemic parents E343 and A26 and the normal offspring.

spring. Photographs of the thyroidectomized parents, E343 and A26, and their normal offspring are shown in figure 7.

While there are many reports on the effects of thyroidectomy upon fertility, no reference other than the report of Petersen *et al.* (4) has been found reporting the lack of normal sexual behavior in the male bovine after removal of the thyroid. The absence of the normal manifestation of estrus in the thyroidectomized female bovine, as observed in the present study, is significant in view of similar behavior in the male bovine. Although basal metabolic measurements were not made, this condition is probably due to a lowered general metabolism, since the administration of thyroid and dinitrophenol restored normal breeding behavior in the bull, E343 (4), and the cow, E294. These observations would seem to indicate that the thyroid may not

be directly concerned with the reproductive processes, but only indirectly through maintenance of an optimum rate of metabolic processes. It is possible that the failure of some dairy cows to show estrus and the slow breeding behavior of some dairy bulls may be due to a hypothyroid condition. The practical implications of this possible relationship justifies further investigation.

SUMMARY

1. The effects of thyroidectomy upon the general appearance and behavior of immature and mature bovine are analogous to those reported for other mammals.

2. Partial thyroidectomy failed to produce any symptoms of hypothyroidism in immature or mature bovine.

3. Growth was definitely retarded by thyroidectomy.

4. Growth was resumed during the late states of pregnancy, probably due to beneficial effects of the thyroid of the developing fetus.

5. The physical manifestations of estrus and male breeding behavior were absent, although the production of fertile ova and sperm occurred, as shown by birth of three normal calves from thyroidectomized parents.

6. Thyroid therapy restored normal breeding behavior in the male and female.

REFERENCES

(1) Andrews, F. N., and Bullard, J. F. The Effects of Partial Thyroidectomy on the Fattening of Steers. Amer. Soc. Anim. Prod. Proc., 112. 1940.

(2) Brody, Samuel, and Frankenbach, R. F. Age Changes in Size, Energy Metabolism and Cardio-Respiratory Activities of Thyroidectomized Cattle. Mo. Agr. Expt. Sta. Res. Bul. 349. 1942.

(3) Koger, Marvin, and Turner, C. W. The Effects of Mild Hyperthyroidism on Growing Animals of Four Species. Mo. Agr. Expt. Sta. Res. Bul. 377. 1943.

(4) Petersen, W. E., Spielman, A. A., Pomeroy, B. S., and Boyd, W. T. Effect of Thyroidectomy upon Sexual Behavior of the Male Bovine. Soc. Expt. Biol. and Med. Proc., 46: 16–17. 1941.

(5) Reineke, E. P., and Turner, C. W. Growth Response of Thyroidectomized Goats to Artificially Formed Thyroprotein. Endocrinology, 29, No. 5: 667–673. 1941.

(6) Simpson, S. The Effect of Thyroidectomy on Growth in Sheep and Goats as Indicated by Body Weight. Quart. Jour. Expt. Physiol., 14: 162–183. 1924.

(7) Spielman, A. A., Petersen, W. E., and Fitch, J. B. The effects of Thyroidectomy on Lactation in the Bovine. Jour. Dairy Sci., 27, No. 6: 441–448. 1944.

FURTHER STUDIES ON THE STABILIZATION OF CAROTENE IN DEHYDRATED ALFALFA AND CEREAL GRASSES

R. C. MILLS AND E. B. HART

From the Department of Biochemistry, University of Wisconsin, Madison

SUPPLEMENTARY NOTE*

More recent experiments in our laboratory show that the carotene of alfalfa and "cerophyl" is stable when subjected to autoclaving at 15 pounds pressure for one hour, when complete displacement of the air by steam vapor is maintained. This initial treatment destroys the carotene oxidase and what destruction of carotene follows on storage is due to "autocatalysis" in the presence of oxygen. On autoclaving the chlorophyll is destroyed and the material becomes gray in color.

After autoclaving in the absence of air and drying both the alfalfa and cerophyl were stored in the dark at room temperature for three months. Samples were also made into pellets ½ by ½ inch and others into larger pellets 4 by 3 inches. Some of the pellets were dipped in flaxo-wax. The alfalfa left loose lost 50% of its carotene in three months, the cerophyl 56%. The small or large pellets of alfalfa lost in the same time about 36%, the cerophyl about 56%. The small alfalfa pellets dipped in flexo-wax lost 36%, the cerophyl 25%. The larger alfalfa pellets dipped in flexo-wax lost 28% in three months, while in the case of the *large* cerophyl flexo-waxed pellets only 0.0–2.0% of the carotene was lost in the same time. It appears then that destruction of the oxidase by autoclaving in the absence of oxygen and further protection of pelleted masses with flexo-wax where the surface is small as compared with the mass of plant tissue, can even at room temperature effect a marked reduction of carotene loss in the materials used.

Apparently the surface of the pellet in reference to the mass of material is of great importance in effecting a stabilization of carotene during storage.

* This note supplements the report which appears in the January 1945 issue pages 1–14.—EDITOR.

TENTATIVE PROGRAM

AMERICAN DAIRY SCIENCE ASSOCIATION

Tuesday, June 12, 1945

9:00–12:00	General Session. Registration. Address of welcome. Presidential address— PRESIDENT A. C. RAGSDALE. Address of guest speaker.
1:00– 4:00	Sectional meetings—Extension Section, Manufacturing Section, and Production Section.
4:00– 5:00	Committee meetings.
7:30	Mixer.

Wednesday, June 13, 1945

9:00–11:00	Sectional meetings.
11:00–12:00	Business meetings of Sections.
1:00– 4:00	Sectional meetings—Manufacturing Section. Joint meeting of Extension and Production Sections on herd health.
4:00– 5:00	Campus tours.
7:30	Reception.

Thursday, June 14, 1945

9:00–11:00	Sectional meetings.
11:00–12:00	Business meetings of Sections.
1:00– 3:00	General Session—Post-war Dairy Development as Affected by Present Research and Trends in Dairy Production and Manufactures. O. E. REED, Bureau of Dairy Industry; L. K. RIGGS, Kraft Cheese Company.
3:00– 4:30	General Business Session.
6:30	Annual Banquet.

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Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

CHEESE

83. Use of Pure Cultures in the Manufacture of Cheddar Cheese. D. D. DEANE, T. G. ANDERSON, F. G. WARREN AND C. D. DAHLE, Pa. State Col., State College, Pa. Natl. Butter and Cheese Jour. *36*, No. 1: 25. Jan. 1945.

A starter from a commercial dairy laboratory, pure cultures of *Streptococcus citrovorus, Streptococcus paracitrovorus, Lactobacillus bulgaricus*, and an unidentified "acidoproteolytic coccus" were used in making Cheddar cheese. The starters did not always produce consistent results. *S. citrovorus* and *paracitrovorus* did not improve the flavor or hasten ripening: *L. bulgaricus* was undesirable; the acidoproteolytic coccus, when used with commercial starter, improved flavor and hastened flavor development without injuring the keeping quality. Curing at 63° F. for 4 weeks and then finishing at 43° F. usually hastened curing but did not always produce as good a flavor as continuous ripening at 43° F. W.V.P.

84. What are the Post-War Possibilities for Packaging Natural Cheese? L. H. JONES, Pliofilm Sales Department, Goodyear Tire and Rubber Co., Akron, Ohio. Natl. Butter and Cheese Jour., *36*, No. 1: 22. Jan. 1945.

There is a demand for natural cheese in packages and sales should be excellent when present restrictions on natural cheese merchandising are removed. The cheese can be cured in consumer-sized units or it can be short-cured in 20-pound blocks then printed and packaged in protective wrappers or special containers. The shelf life of packages of different types will vary but should be effective for 60 to 90 days. Some packages can be spoiled by rough handling, strong light, and exposure to heat so the retailer should be educated to treat them properly and to merchandise them intelligently. W.V.P.

DISEASE

85. Research on Brucellosis in Latin America. EDITORIAL, Amer. Med. Assoc. Jour., *126*, No. 15: 962. Dec. 9, 1944.

Two recently published monographs are briefly reviewed with praise for their merit: Brucelosis, Mexico, D. F., Ediciones de la Revista medicina, 1942, by Dr. M. Ruiz Castañeda; and, Brucelosis: Estudio de esta enfermedad en el Uruguay, Montevideo, Editorial Independencia, 1944, by Drs. P. Purriel, R. Risso, and J. Espasandin. A few interesting facts are stated:

In Uruguay the melitensis variety is absent whereas the abortus variety

is quite prevalent; morbidity for the population at large is low, mortality is nil. The disease is of a professional type. Milk-producing cows show 50% infection.

In Mexico 95.2% of human cases are of the melitensis variety. Goat infection is suspected to be high; that of cattle varies from 25 to 50%; that of swine is about 35%. At least 3% of the human population has been or is infected. D.P.G.

FOOD VALUE OF DAIRY PRODUCTS

86. Dairy Products Promote Health and Economic Welfare. J. H. FRANDSEN, Mass. State Col., Amherst, Mass. Milk Dealer, *33*, No. 12: 54–55. Sept. 1944.

Dairy products are acknowledged to be essential in all adequate diets not only to maintain but to increase our nutritional health and well-being and to prevent malnutrition. The author raises the question of relative nutritive value of oleomargarine and butter, even when oleomargarine is vitamin-fortified, and cites experimental data to show that the question might still be considered debatable. He recommends that existing legislation to insure consumer protection be continued in order to differentiate between oleomargarine and butter. "It is a well-known fact that a marked decrease in butter sales depresses" all dairy products and reduces milk production, thereby bringing about soil depletion and loss of fertility through lessened livestock population. There should be no need to limit the production of milk and dairy products. C.S.T.

87. Evaporated Milk in Infant Feeding. An Evaluation With and Without Added Carbohydrate. H. R. LITCHFIELD. Arch. Ped., *61:* 12. Dec. 1944.

Analysis of results from a study of 195 cases is convincing that newborn infants during the first ten days on evaporated milk formulas with no added carbohydrates can be expected to lose weight. Even a more concentrated formula without added carbohydrate is not effective in the period of study to accomplish gain in weight. Addition of carbohydrate to the evaporated milk formula on the other hand favors weight gain in most instances.

Special attention is warranted to the gains in weight of the newborn infants on Biolac (a modified milk with added lactose, vitamins and iron) in the first ten-day period. Biolac-fed infants received a lower caloric intake than those on evaporated milk formula with added carbohydrate. Perhaps the difference in weight response may be accounted for in the extra vitamin and iron content of the Biolac or in the fact that the added carbohydrate in Biolac is lactose.

Studies on older infants also show that need for carbohydrates in infant feeding is unquestionable.

The author recommends that newborn infants and those in early infancy be fed milk formulas with additional carbohydrates and vitamin B_1 for optimal growth and development. H.P.

ICE CREAM

88. Manufacture of Ice Cream with Limited Milk Solids. T. R. FREEMAN AND E. L. FOUTS, Florida Agr. Expt. Sta., Bul. 402. Oct. 1944.

Various flours and other products were added to ice cream as a means of aiding the body and texture during the war period of curtailed milk solids. Certain products such as corn starch, cottonseed flour, soya flour, peanut flour and peanut proteins were unsuitable because of flavor defects. Oat flour up to two per cent (2%) proved to be satisfactory from a flavor and texture standpoint.

In general, wheat flour was found to be most satisfactory because of price availability effect on body, texture and flavor.

Ice cream of nine per cent (9%) serum solids plus two per cent (2%) flour, eight per cent (8%) serum solids, plus two per cent (2%) flour, and seven per cent (7%) serum solids plus two per cent (2%) flour compared favorably in body and texture with ice creams containing eleven (11), ten (10), and nine per cent (9%) serum solids respectively.

Stabilizer could be eliminated if 2–2.5 per cent flour was added to a mix containing 10.5 per cent fat, 14 per cent sugar and 8.5 per cent serum solids.

It is recommended that 0.10 per cent salt be added to mixes containing two (2) per cent flour and less than 10 per cent serum solids. Wheat flour mixes should be pasteurized at a temperature of 155–160° F. for 30 minutes. Wheat flour is readily incorporated when mixed with 2–3 times its weight of sugar and added to the liquid materials at a temperature between 100–130° F. C.D.D.

89. A Technique for Testing Consumer Preferences, with Special Reference to the Constituents of Ice Cream. C. I. BLISS, E. O. ANDERSON AND R. E. MARLAND, University of Connecticut, Storrs, Connecticut. Storrs Agr. Expt. Sta. Bul., 251. Nov. 1943.

Consumer preferences for two qualitative factors in ice cream, vanilla and chocolate flavoring, and for two quantitative factors, percentage concentration of serum solids and of fat, have been tested on college students. The subjects ranked the four alternative ice creams of each series in order of choice. These ranks were then converted to scores suitable for use in the analysis of variance, with which the significant consumer preferences were identified. American process chocolate was preferred to three Dutch types, which were not scored equally. Natural vanilla flavoring rated higher than the artificial product, but a 50–50 mixture of the two scored highest of all.

In ice cream containing 14 per cent fat, the preferred concentration of serum solids was 10.17 ± 0.37 per cent; in that containing 11 per cent of serum solids, the preferred concentration of fat was 13.49 ± 0.23 per cent. Both sexes concurred in these results.

The design and statistical analysis of choice tests are given in detail. Simple methods are described for computing the concentration giving the maximum score and its error and for testing the homogeneity of the response to different items in a series. Possible applications of the technique are indicated. C.D.D.

MILK

90. Suggestions for Reducing Reinspections and Suspensions of Milk Producers. HARLOW L. PENDLETON, Dairy Inspector, Mass. State Dept. Agr. Milk Dealer, *33*, No. 12: 38–39, 96–99. Sept. 1944.

A survey of 10 Vermont country plants in 1941 and 1942 to show the extent the various items in dairy farm inspection scores contribute to the approval, non-approval, or suspension of dairy farms, was the basis of this study. The purpose was (1) to check items found to be in violation; (2) to determine items contributing to suspensions; and (3) to make specific recommendations for improving major items entering into proper production methods. A standard system of scoring was used with all farms studied, covering 1543 farms in 1941 and 1498 farms in 1942, and stressing the two big "C's" of sanitary milk production—cleanliness and cooling. Approximately 80% of all farms inspected were approved. A copy of standard form used is shown. Cleanliness of the dairy herd, including use of non-absorbent bedding, clipping of animals, disposal of manure, stable sanitation, cleanliness of milk rooms and of utensils by use of proper cleaners, are the prime essentials in the production of clean milk.

Proper cooling of clean milk includes knowledge of cooling temperatures required, immediate cooling, adequate cold water supply, cooling tanks, and location of milk room. These major items contribute to greatest percentage of suspensions through violations. Percentages of violations and suspensions together with recommendations for correction and improvement are tabulated. By observance of recommendations not over 10% of non-approvals and not over 1% of suspensions, instead of 20% and 6.28%, respectively, should be necessary. C.S.T.

MISCELLANEOUS

91. Outlook for Aluminum in the Dairy Industry. ANONYMOUS. Milk Dealer, *33*, No. 11: 41–42, 84–88. Aug. 1944.

The use of aluminum in the dairy plant as a post-war product, due to war-time developments and improvements in methods of utilization and

processing should be wide-spread. These changes have resulted in a 25% reduction in costs in utilization and in methods of welding, riveting, brazing and other fabricating processes—all capable of being adapted to its use in post-war dairy equipment. The newer knowledge of caring for and cleaning aluminum used in war-time utilization when applied to the dairy machinery industry should enhance aluminum's adaptation to the dairy industry from aluminum churns to aluminum foil for wrapping butter and cheese and from milk pails to milk trucks. The problem of corrosion through use of strong alkalies and both direct and indirect galvanic action between different metals or by dissolved metals has largely been overcome as have the problem of proper, readily cleaned joints, valves and welds and use of alloys.

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(b) ..... **Construct the Holland Tunnel under the Hudson River?** — 3 years / 7 years

(c) ..... **Build the first 100,000 miles of railroad?** — 25 years / 50 years

(d) ..... **Educate 8 of 10 people to know that dextrose sugar is energy in its purest form?** — 6 years / 12 years

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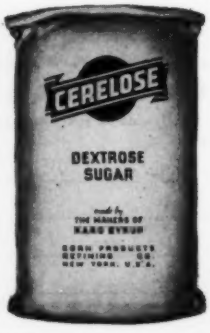

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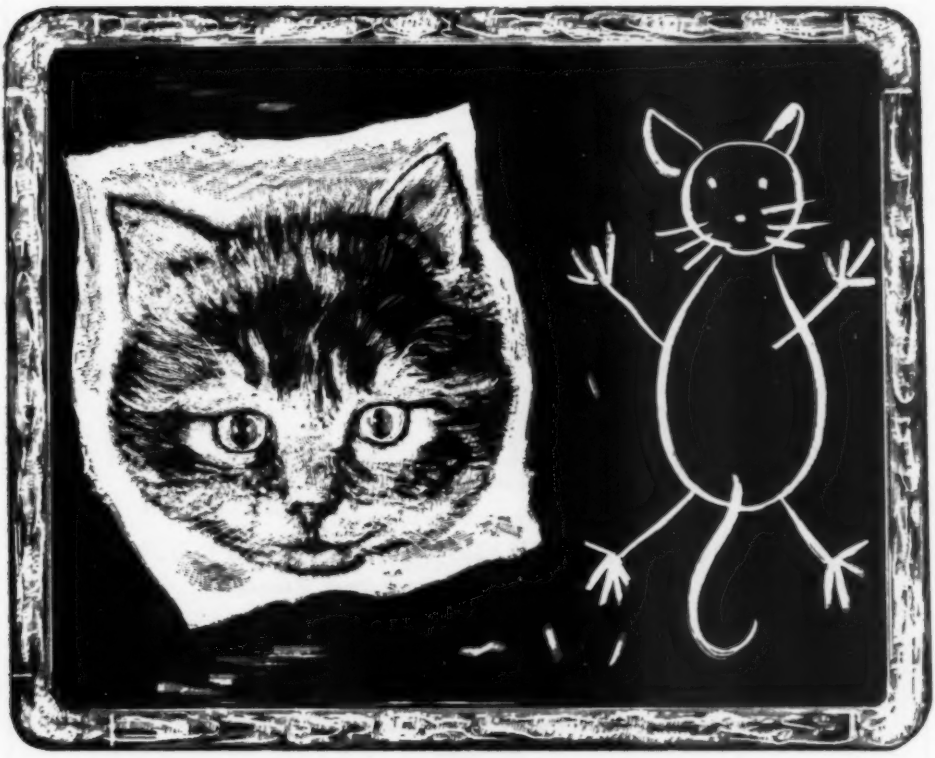

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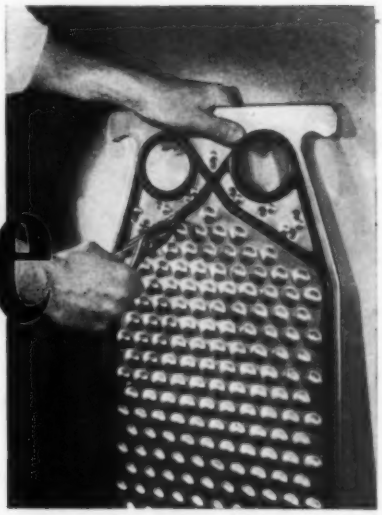

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